



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | | |
|---|--|----|--|
| (51) International Patent Classification ⁶ : C12N 15/12, C07K 14/47, A61K 38/17, C07K 16/18, C12Q 1/68, C12N 15/62, 15/11, G01N 33/50 | | A1 | (11) International Publication Number: WO 98/41628 |
| | | | (43) International Publication Date: 24 September 1998 (24.09.98) |
| (21) International Application Number: PCT/US98/05255 | | | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). |
| (22) International Filing Date: 18 March 1998 (18.03.98) | | | |
| (30) Priority Data: 60/041,263 19 March 1997 (19.03.97) US | | | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). |
| (71) Applicant: ZYMOGENETICS, INC. [US/US]; 1201 Eastlake Avenue East, Seattle, WA 98102 (US). | | | |
| (72) Inventor: SHEPPARD, Paul, O.; 20717 N.E. 2nd Street, Redmond, WA 98053 (US). | | | |
| (74) Agent: LINGENFELTER, Susan, E.; ZymoGenetics, Inc., 1201 Eastlake Avenue East, Seattle, WA 98102 (US). | | | |

(54) Title: SECRETED SALIVARY ZSIG32 POLYPEPTIDES

(57) Abstract

The present invention relates to polynucleotide and polypeptide molecules for secreted salivary zsig32 polypeptides. The polypeptides, and polynucleotides encoding them modulate adhesion or modulate or indicate salivary gland function. The present invention also includes antibodies and binding proteins for the zsig32 polypeptides.

Published

*With international search report.**Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|--|----|--|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | ML | Mali | TR | Turkey |
| BG | Bulgaria | HU | Hungary | MN | Mongolia | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MR | Mauritania | UA | Ukraine |
| BR | Brazil | IL | Israel | MW | Malawi | UG | Uganda |
| BY | Belarus | IS | Iceland | MX | Mexico | US | United States of America |
| CA | Canada | IT | Italy | NE | Niger | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NL | Netherlands | VN | Viet Nam |
| CG | Congo | KE | Kenya | NO | Norway | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NZ | New Zealand | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | PL | Poland | | |
| CM | Cameroon | KR | Republic of Korea | PT | Portugal | | |
| CN | China | KZ | Kazakhstan | RO | Romania | | |
| CU | Cuba | LC | Saint Lucia | RU | Russian Federation | | |
| CZ | Czech Republic | LI | Liechtenstein | SD | Sudan | | |
| DE | Germany | LK | Sri Lanka | SE | Sweden | | |
| DK | Denmark | LR | Liberia | SG | Singapore | | |
| EE | Estonia | | | | | | |

5

DESCRIPTION

SECRETED SALIVARY ZSIG32 POLYPEPTIDES

BACKGROUND OF THE INVENTION

10 The salivary glands synthesize and secrete a number of proteins having diverse biological functions. Such proteins facilitate lubrication of the oral cavity (e.g., mucins and proline-rich proteins), remineralization (e.g., statherin and ionic proline-rich proteins) and 15 digestion (e.g., amylase, lipase and proteases) and provide anti-microbial (e.g., proline-rich proteins, lysozyme, histatins and lactoperoxidase) and mucosal integrity maintenance (e.g., mucins) capabilities. In addition, saliva is a rich source of growth factors 20 synthesized by the salivary glands. For example, saliva is known to contain epidermal growth factor (EGF), nerve growth factor (NGF), transforming growth factor-alpha (TGF- α), transforming growth factor-beta (TGF- β), insulin, insulin-like growth factors I and II (IGF-I and IGF-II) 25 and fibroblast growth factor (FGF). See, for example, Zelles et al., J. Dental. Res. 74(12): 1826-32, 1995. Synthesis of growth factors by the salivary gland is believed to be androgen-dependent and to be necessary for the health of the oral cavity and gastrointestinal tract.

30 Some salivary gland-produced proteins, such as EGF, are believed to have systemic wound healing effects. Effective wound healing appears to require extended

acting to capture saliva growth factors. Also, combinations of growth factors, such as those found in

saliva, may be necessary for optimal wound healing. Moreover, protease inhibitors, which are also produced by the salivary glands, appear to facilitate growth factor activity.

5 In addition, saliva contains adhesive proteins having protective properties with regard to infection by exogenous microorganisms. Such adhesive proteins bind exogenous microorganisms and facilitate the degradation or expulsion thereof. From this rich source of biologically 10 relevant proteins, new secreted proteins are sought. Also, given the importance and variety of saliva proteins, conditions involving inadequate saliva production or secretion have inspired investigative effort.

Rattus norvegicus common salivary protein 1 15 (U00964_1) and a murine homolog of common salivary protein 1 (S76879_1) have been discovered and characterized. See, for example, Girard et al., J. Biol. Chem. 268(35): 26592-601, 1993. Common salivary protein 1 is so designated as a result of the expression thereof in cells of all major 20 salivary glands. Evidence exists that such expression is androgen-regulated in the rat submandibular gland. Common salivary protein 1 does not include structural features associated with many other salivary proteins, including tandemly repeated sequences, a high density of charged 25 residues and/or an unusually large proportion of a few amino acids. Common salivary protein 1 is somewhat homologous to spermine binding proteins discussed below, but lacks the highly acidic carboxy terminal domain 30 thereof. Thus, the proteins may be evolutionarily related without being functionally related.

35 Salivary glands share significant features with other glands, such as the prostate gland. For example, the salivary glands and prostate gland are classified as slow replicators with respect to their proliferative capacity. See, for example, Zajicek, Med. Hypotheses 7(10): 1241-51, 1981. Such slow replicators exhibit

similar onogenies and proceed during regeneration and neoplasia through similar stages. The prostate gland also appears to produce growth factors, such as EGF and NGF, and other biologically important proteins, such as 5 kallikreins. See, for example, Hiramatsu et al., Biochem. Int. 17(2): 311-7, 1988, Harper et al., J. Biol. Chem. 257(14): 8541-8, 1982 and Brady et al., Biochemistry 28(12): 5203-10, 1988. Prostate gland function also appears to be androgen-dependent. Consequently, proteins 10 associated with the prostate gland are also sought.

Glandular function is believed to be androgen-dependent. Expression of secreted glycoproteins, having spermine-binding activity, by mouse and rat prostate has also been postulated to be androgen-dependent. See, for 15 example, Mills et al., Nucleic Acids Res. 15: 7709-24, 1987. Spermine-binding protein mRNA expression appears to be induced by exposure to androgens, with an increase therein by 2-3 fold being observed within 16 hours and continuing for several days. Thus, intracellular levels 20 of specific hormone-dependent mRNA, such as spermine binding protein mRNA, are useful markers of hormone action. See, Labrie et al., Endocrinology 124(6): 2745-54, 1989. Spermine-binding protein is also useful for studying cAMP-independent protein kinases, because the 25 protein is under androgenic control through the action of such kinases. See, for example, Goueli et al., Biochem. J. (England) 230(2): 293-302, 1985.

Spermine-binding proteins bind to polyamines, such as spermine. Prostatic fluid, for example, is rich 30 in polyamines, and spermine-binding proteins have therefore been postulated to serve as carriers of such polyamines in the seminal fluid. Spermine-binding

For example, Maekawa, Mie. Med. 39(2): 221-5, 1989. In addition, spermine binds to and is believed to

modulate the activity of the N-methyl-D-aspartate (NMDA) receptor, a ligand-gated ion channel (Bergeron et al., J. Med. Chem. **38**: 425-8, 1995). Also, spermine is believed to effect vascular smooth muscle cell contractility via 5 inhibition of myosin phosphatase (Sward et al., Am. J. Physiology **269**(3 pt. 1): C563-71, 1995). Thus, homologs of spermine-binding proteins are sought.

The present invention provides such polypeptides for these and other uses that should be apparent to those 10 skilled in the art from the teachings herein.

SUMMARY OF THE INVENTION

Within one aspect the invention provides an isolated polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 23-178 of SEQ ID NO:2. Within one embodiment the polypeptide is at least 90% identical in amino acid sequence to residues 23-178 of SEQ ID NO:2. Within another embodiment the polypeptide comprises 20 residues 27-178 of SEQ ID NO:2. Within yet another embodiment the polypeptide comprises residues 7-178 of SEQ ID NO:2. Within still another embodiment the polypeptide comprises residues 1-178 of SEQ ID NO:2. Within another embodiment the polypeptide is at least 1 kb in length. Within yet another embodiment the polypeptide is 25 covalently linked to a moiety selected from the group consisting of affinity tags, toxins, radionucleotides, enzymes and fluorophores. Within a related embodiment the moiety is an affinity tag selected from the group consisting of polyhistidine, FLAG, Glu-Glu, glutathione S 30 transferase and an immunoglobulin heavy chain constant region. Within another related embodiment the polypeptide further comprises a proteolytic cleavage site between the sequence of amino acid residues and the affinity tag.

35 Within another aspect is provided an expression vector comprising the following operably linked elements:

a transcription promoter; a DNA segment encoding a polypeptide as described above; and a transcriptional terminator. Within a related embodiment the DNA segment encodes a polypeptide covalently linked to an affinity tag 5 selected from the group consisting of polyhistidine, FLAG, Glu-Glu, glutathione S transferase and an immunoglobulin heavy chain constant region. Within a related embodiment the DNA further encodes a secretory signal sequence operably linked to said polypeptide. Within another 10 embodiment the secretory signal sequence encodes residues 7-22 of SEQ ID NO:2. Within a related embodiment the secretory signal sequence encodes residues 1-22 of SEQ ID NO:2. Within another related embodiment is provided a cultured cell into which has been introduced an expression 15 vector as described above, wherein the cell expresses the polypeptide encoded by the DNA segment.

Within another aspect is provided a method of producing a protein comprising: culturing a cell into which has been introduced an expression vector as 20 described above, whereby the cell expresses the protein encoded by the DNA segment; and recovering the expressed protein.

Within another aspect is provided a pharmaceutical composition comprising a polypeptide as 25 described above in combination with a pharmaceutically acceptable vehicle.

Within other aspects are provided an antibody that specifically binds to an epitope of a polypeptide as described above and a binding protein that specifically 30 binds to an epitope of a polypeptide as described above.

Within a further aspect is provided an isolated

35 polynucleotide is selected from the group consisting of, a sequence of nucleotides from nucleotide 169 to

nucleotide 704 of SEQ ID NO:1; b) a sequence of nucleotides from nucleotide 186 to nucleotide 704 of SEQ ID NO:2; c) a sequence of nucleotides from nucleotide 234 to nucleotide 704 of SEQ ID NO:2; d) a sequence of 5 nucleotides from nucleotide 246 to nucleotide 704 of SEQ ID NO:2; e) allelic variants of a), b), c) or d); and f) nucleotide sequences complementary to a), b), c), d) or e). Within another embodiment the polynucleotide is from 471 to 853 nucleotides in length. Within another 10 embodiment the polynucleotide comprises nucleotide 1 to nucleotide 534 of SEQ ID NO:20. Within another embodiment the polynucleotide is DNA.

Within another aspect of the invention is provided an oligonucleotide probe or primer comprising 14 15 contiguous nucleotides of a polynucleotide of SEQ ID NO:20 or a sequence complementary to SEQ ID NO:20.

Within yet another aspect is provided a method for detecting a genetic abnormality in a patient, comprising: obtaining a genetic sample from a patient; 20 incubating the genetic sample with a polynucleotide comprising at least 14 contiguous nucleotides of SEQ ID NO:1 or the complement of SEQ ID NO:1, under conditions wherein said polynucleotide will hybridize to complementary polynucleotide sequence, to produce a first 25 reaction product; comparing said first reaction product to a control reaction product, wherein a difference between said first reaction product and said control reaction product is indicative of a genetic abnormality in the patient.

30 Within a further aspect is provided a DNA construct encoding a polypeptide fusion, said fusion comprising a secretory signal sequence selected from the group consisting of: (a) amino acid residues 1-22 of SEQ ID NO:2; and (b) amino acid residues 7-22 of SEQ ID NO:2; 35 wherein the secretory signal sequence is operably linked to an additional polypeptide.

Within another aspect is provided a method for detecting zsig32 polypeptides comprising: exposing a polypeptide containing sample to an antibody attached to a solid support, wherein said antibody binds to an epitope 5 of a zsig32 polypeptide; washing said immobilized antibody-polypeptide to remove unbound contaminants; exposing the immobilized antibody-polypeptide to a second antibody directed to a second epitope of a zsig32 polypeptide, wherein the second antibody is associated 10 with a detectable label; and detecting the detectable label.

These and other aspects of the invention will become evident upon reference to the following detailed description of the invention and the attached drawing.

15

BRIEF DESCRIPTION OF THE DRAWING

The Figure illustrates an alignment of mouse ventral prostate spermine-binding protein (SPBP_MOUSE), *Rattus norvegicus* common salivary protein 1 (U00964_1), a 20 murine common salivary protein 1 (S76879_1) and a zsig32 polypeptide of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

25 Prior to setting forth the invention in detail, it may be helpful to the understanding thereof to define the following terms:

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second 30 polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of a detectable label.

Affinity tags include a poly-histidine tract, protein A (Nilsson et al., EMBO J. 4:1075, 1985; Nilsson et al.,

Methods Enzymol. 198:3, 1991), glutathione S transferase (Smith and Johnson, Gene 67:31, 1988), Glu-Glu affinity tag (Grussenmeyer et al., Proc. Natl. Acad. Sci. USA 82:7952-4, 1985), substance P, Flag™ peptide (Hopp et al., 5 Biotechnology 6:1204-10, 1988), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford et al., Protein Expression and Purification 2: 95-107, 1991. DNAs encoding affinity tags are available from commercial suppliers (e.g., Pharmacia 10 Biotech, Piscataway, NJ).

The term "allelic variant" denotes any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic 15 polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

20 The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides and proteins. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide or protein to denote 25 proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a protein is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete 30 protein.

The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are 35 prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include

receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the 5 complement/anti-complement pair preferably has a binding affinity of $<10^9 \text{ M}^{-1}$.

The term "complements of polynucleotide molecules" denotes polynucleotide molecules having a complementary base sequence and reverse orientation as 10 compared to a reference sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or 15 more degenerate codons (as compared to a reference polynucleotide molecule that encodes a polypeptide). Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

20 The term "expression vector" denotes a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments may include promoter and 25 terminator sequences, and may optionally include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, and the like. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

30 The term "isolated", when applied to a polynucleotide molecule, denotes that the polynucleotide

10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400 405 410 415 420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495 500 505 510 515 520 525 530 535 540 545 550 555 560 565 570 575 580 585 590 595 600 605 610 615 620 625 630 635 640 645 650 655 660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 905 910 915 920 925 930 935 940 945 950 955 960 965 970 975 980 985 990 995 1000 1005 1010 1015 1020 1025 1030 1035 1040 1045 1050 1055 1060 1065 1070 1075 1080 1085 1090 1095 1100 1105 1110 1115 1120 1125 1130 1135 1140 1145 1150 1155 1160 1165 1170 1175 1180 1185 1190 1195 1200 1205 1210 1215 1220 1225 1230 1235 1240 1245 1250 1255 1260 1265 1270 1275 1280 1285 1290 1295 1300 1305 1310 1315 1320 1325 1330 1335 1340 1345 1350 1355 1360 1365 1370 1375 1380 1385 1390 1395 1400 1405 1410 1415 1420 1425 1430 1435 1440 1445 1450 1455 1460 1465 1470 1475 1480 1485 1490 1495 1500 1505 1510 1515 1520 1525 1530 1535 1540 1545 1550 1555 1560 1565 1570 1575 1580 1585 1590 1595 1600 1605 1610 1615 1620 1625 1630 1635 1640 1645 1650 1655 1660 1665 1670 1675 1680 1685 1690 1695 1700 1705 1710 1715 1720 1725 1730 1735 1740 1745 1750 1755 1760 1765 1770 1775 1780 1785 1790 1795 1800 1805 1810 1815 1820 1825 1830 1835 1840 1845 1850 1855 1860 1865 1870 1875 1880 1885 1890 1895 1900 1905 1910 1915 1920 1925 1930 1935 1940 1945 1950 1955 1960 1965 1970 1975 1980 1985 1990 1995 2000 2005 2010 2015 2020 2025 2030 2035 2040 2045 2050 2055 2060 2065 2070 2075 2080 2085 2090 2095 2100 2105 2110 2115 2120 2125 2130 2135 2140 2145 2150 2155 2160 2165 2170 2175 2180 2185 2190 2195 2200 2205 2210 2215 2220 2225 2230 2235 2240 2245 2250 2255 2260 2265 2270 2275 2280 2285 2290 2295 2300 2305 2310 2315 2320 2325 2330 2335 2340 2345 2350 2355 2360 2365 2370 2375 2380 2385 2390 2395 2400 2405 2410 2415 2420 2425 2430 2435 2440 2445 2450 2455 2460 2465 2470 2475 2480 2485 2490 2495 2500 2505 2510 2515 2520 2525 2530 2535 2540 2545 2550 2555 2560 2565 2570 2575 2580 2585 2590 2595 2600 2605 2610 2615 2620 2625 2630 2635 2640 2645 2650 2655 2660 2665 2670 2675 2680 2685 2690 2695 2700 2705 2710 2715 2720 2725 2730 2735 2740 2745 2750 2755 2760 2765 2770 2775 2780 2785 2790 2795 2800 2805 2810 2815 2820 2825 2830 2835 2840 2845 2850 2855 2860 2865 2870 2875 2880 2885 2890 2895 2900 2905 2910 2915 2920 2925 2930 2935 2940 2945 2950 2955 2960 2965 2970 2975 2980 2985 2990 2995 3000 3005 3010 3015 3020 3025 3030 3035 3040 3045 3050 3055 3060 3065 3070 3075 3080 3085 3090 3095 3100 3105 3110 3115 3120 3125 3130 3135 3140 3145 3150 3155 3160 3165 3170 3175 3180 3185 3190 3195 3200 3205 3210 3215 3220 3225 3230 3235 3240 3245 3250 3255 3260 3265 3270 3275 3280 3285 3290 3295 3300 3305 3310 3315 3320 3325 3330 3335 3340 3345 3350 3355 3360 3365 3370 3375 3380 3385 3390 3395 3400 3405 3410 3415 3420 3425 3430 3435 3440 3445 3450 3455 3460 3465 3470 3475 3480 3485 3490 3495 3500 3505 3510 3515 3520 3525 3530 3535 3540 3545 3550 3555 3560 3565 3570 3575 3580 3585 3590 3595 3600 3605 3610 3615 3620 3625 3630 3635 3640 3645 3650 3655 3660 3665 3670 3675 3680 3685 3690 3695 3700 3705 3710 3715 3720 3725 3730 3735 3740 3745 3750 3755 3760 3765 3770 3775 3780 3785 3790 3795 3800 3805 3810 3815 3820 3825 3830 3835 3840 3845 3850 3855 3860 3865 3870 3875 3880 3885 3890 3895 3900 3905 3910 3915 3920 3925 3930 3935 3940 3945 3950 3955 3960 3965 3970 3975 3980 3985 3990 3995 4000 4005 4010 4015 4020 4025 4030 4035 4040 4045 4050 4055 4060 4065 4070 4075 4080 4085 4090 4095 4100 4105 4110 4115 4120 4125 4130 4135 4140 4145 4150 4155 4160 4165 4170 4175 4180 4185 4190 4195 4200 4205 4210 4215 4220 4225 4230 4235 4240 4245 4250 4255 4260 4265 4270 4275 4280 4285 4290 4295 4300 4305 4310 4315 4320 4325 4330 4335 4340 4345 4350 4355 4360 4365 4370 4375 4380 4385 4390 4395 4400 4405 4410 4415 4420 4425 4430 4435 4440 4445 4450 4455 4460 4465 4470 4475 4480 4485 4490 4495 4500 4505 4510 4515 4520 4525 4530 4535 4540 4545 4550 4555 4560 4565 4570 4575 4580 4585 4590 4595 4600 4605 4610 4615 4620 4625 4630 4635 4640 4645 4650 4655 4660 4665 4670 4675 4680 4685 4690 4695 4700 4705 4710 4715 4720 4725 4730 4735 4740 4745 4750 4755 4760 4765 4770 4775 4780 4785 4790 4795 4800 4805 4810 4815 4820 4825 4830 4835 4840 4845 4850 4855 4860 4865 4870 4875 4880 4885 4890 4895 4900 4905 4910 4915 4920 4925 4930 4935 4940 4945 4950 4955 4960 4965 4970 4975 4980 4985 4990 4995 5000 5005 5010 5015 5020 5025 5030 5035 5040 5045 5050 5055 5060 5065 5070 5075 5080 5085 5090 5095 5100 5105 5110 5115 5120 5125 5130 5135 5140 5145 5150 5155 5160 5165 5170 5175 5180 5185 5190 5195 5200 5205 5210 5215 5220 5225 5230 5235 5240 5245 5250 5255 5260 5265 5270 5275 5280 5285 5290 5295 5300 5305 5310 5315 5320 5325 5330 5335 5340 5345 5350 5355 5360 5365 5370 5375 5380 5385 5390 5395 5400 5405 5410 5415 5420 5425 5430 5435 5440 5445 5450 5455 5460 5465 5470 5475 5480 5485 5490 5495 5500 5505 5510 5515 5520 5525 5530 5535 5540 5545 5550 5555 5560 5565 5570 5575 5580 5585 5590 5595 5600 5605 5610 5615 5620 5625 5630 5635 5640 5645 5650 5655 5660 5665 5670 5675 5680 5685 5690 5695 5700 5705 5710 5715 5720 5725 5730 5735 5740 5745 5750 5755 5760 5765 5770 5775 5780 5785 5790 5795 5800 5805 5810 5815 5820 5825 5830 5835 5840 5845 5850 5855 5860 5865 5870 5875 5880 5885 5890 5895 5900 5905 5910 5915 5920 5925 5930 5935 5940 5945 5950 5955 5960 5965 5970 5975 5980 5985 5990 5995 6000 6005 6010 6015 6020 6025 6030 6035 6040 6045 6050 6055 6060 6065 6070 6075 6080 6085 6090 6095 6100 6105 6110 6115 6120 6125 6130 6135 6140 6145 6150 6155 6160 6165 6170 6175 6180 6185 6190 6195 6200 6205 6210 6215 6220 6225 6230 6235 6240 6245 6250 6255 6260 6265 6270 6275 6280 6285 6290 6295 6300 6305 6310 6315 6320 6325 6330 6335 6340 6345 6350 6355 6360 6365 6370 6375 6380 6385 6390 6395 6400 6405 6410 6415 6420 6425 6430 6435 6440 6445 6450 6455 6460 6465 6470 6475 6480 6485 6490 6495 6500 6505 6510 6515 6520 6525 6530 6535 6540 6545 6550 6555 6560 6565 6570 6575 6580 6585 6590 6595 6600 6605 6610 6615 6620 6625 6630 6635 6640 6645 6650 6655 6660 6665 6670 6675 6680 6685 6690 6695 6700 6705 6710 6715 6720 6725 6730 6735 6740 6745 6750 6755 6760 6765 6770 6775 6780 6785 6790 6795 6800 6805 6810 6815 6820 6825 6830 6835 6840 6845 6850 6855 6860 6865 6870 6875 6880 6885 6890 6895 6900 6905 6910 6915 6920 6925 6930 6935 6940 6945 6950 6955 6960 6965 6970 6975 6980 6985 6990 6995 7000 7005 7010 7015 7020 7025 7030 7035 7040 7045 7050 7055 7060 7065 7070 7075 7080 7085 7090 7095 7100 7105 7110 7115 7120 7125 7130 7135 7140 7145 7150 7155 7160 7165 7170 7175 7180 7185 7190 7195 7200 7205 7210 7215 7220 7225 7230 7235 7240 7245 7250 7255 7260 7265 7270 7275 7280 7285 7290 7295 7300 7305 7310 7315 7320 7325 7330 7335 7340 7345 7350 7355 7360 7365 7370 7375 7380 7385 7390 7395 7400 7405 7410 7415 7420 7425 7430 7435 7440 7445 7450 7455 7460 7465 7470 7475 7480 7485 7490 7495 7500 7505 7510 7515 7520 7525 7530 7535 7540 7545 7550 7555 7560 7565 7570 7575 7580 7585 7590 7595 7600 7605 7610 7615 7620 7625 7630 7635 7640 7645 7650 7655 7660 7665 7670 7675 7680 7685 7690 7695 7700 7705 7710 7715 7720 7725 7730 7735 7740 7745 7750 7755 7760 7765 7770 7775 7780 7785 7790 7795 7800 7805 7810 7815 7820 7825 7830 7835 7840 7845 7850 7855 7860 7865 7870 7875 7880 7885 7890 7895 7900 7905 7910 7915 7920 7925 7930 7935 7940 7945 7950 7955 7960 7965 7970 7975 7980 7985 7990 7995 8000 8005 8010 8015 8020 8025 8030 8035 8040 8045 8050 8055 8060 8065 8070 8075 8080 8085 8090 8095 8100 8105 8110 8115 8120 8125 8130 8135 8140 8145 8150 8155 8160 8165 8170 8175 8180 8185 8190 8195 8200 8205 8210 8215 8220 8225 8230 8235 8240 8245 8250 8255 8260 8265 8270 8275 8280 8285 8290 8295 8300 8305 8310 8315 8320 8325 8330 8335 8340 8345 8350 8355 8360 8365 8370 8375 8380 8385 8390 8395 8400 8405 8410 8415 8420 8425 8430 8435 8440 8445 8450 8455 8460 8465 8470 8475 8480 8485 8490 8495 8500 8505 8510 8515 8520 8525 8530 8535 8540 8545 8550 8555 8560 8565 8570 8575 8580 8585 8590 8595 8600 8605 8610 8615 8620 8625 8630 8635 8640 8645 8650 8655 8660 8665 8670 8675 8680 8685 8690 8695 8700 8705 8710 8715 8720 8725 8730 8735 8740 8745 8750 8755 8760 8765 8770 8775 8780 8785 8790 8795 8800 8805 8810 8815 8820 8825 8830 8835 8840 8845 8850 8855 8860 8865 8870 8875 8880 8885 8890 8895 8900 8905 8910 8915 8920 8925 8930 8935 8940 8945 8950 8955 8960 8965 8970 8975 8980 8985 8990 8995 9000 9005 9010 9015 9020 9025 9030 9035 9040 9045 9050 9055 9060 9065 9070 9075 9080 9085 9090 9095 9100 9105 9110 9115 9120 9125 9130 9135 9140 9145 9150 9155 9160 9165 9170 9175 9180 9185 9190 9195 9200 9205 9210 9215 9220 9225 9230 9235 9240 9245 9250 9255 9260 9265 9270 9275 9280 9285 9290 9295 9300 9305 9310 9315 9320 9325 9330 9335 9340 9345 9350 9355 9360 9365 9370 9375 9380 9385 9390 9395 9400 9405 9410 9415 9420 9425 9430 9435 9440 9445 9450 9455 9460 9465 9470 9475 9480 9485 9490 9495 9500 9505 9510 9515 9520 9525 9530 9535 9540 9545 9550 9555 9560 9565 9570 9575 9580 9585 9590 9595 9600 9605 9610 9615 9620 9625 9630 9635 9640 9645 9650 9655 9660 9665 9670 9675 9680 9685 9690 9695 9700 9705 9710 9715 9720 9725 9730 9735 9740 9745 9750 9755 9760 9765 9770 9775 9780 9785 9790 9795 9800 9805 9810 9815 9820 9825 9830 9835 9840 9845 9850 9855 9860 9865 9870 9875 9880 9885 9890 9895 9900 9905 9910 9915 9920 9925 9930 9935 9940 9945 9950 9955 9960 9965 9970 9975 9980 9985 9990 9995 9999

natural environment and include cDNA and genomic clones. Isolated DNA molecules of the present invention are free of other genes with which they are ordinarily associated, but may include naturally occurring 5' and 3' untranslated regions such as promoters and terminators. The identification of associated regions will be evident to one of ordinary skill in the art (see for example, Dynan and Tijan, Nature 316:774-78, 1985). When applied to a protein, the term "isolated" indicates that the protein is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated protein is substantially free of other proteins, particularly other proteins of animal origin. It is preferred to provide the protein in a highly purified form, i.e., greater than 95% pure, more preferably greater than 99% pure.

The term "operably linked", when referring to DNA segments, denotes that the segments are arranged so that they function in concert for their intended purposes, e.g. transcription initiates in the promoter and proceeds through the coding segment to the terminator.

The term "ortholog" denotes a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

"Paralogs" are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, α -globin, β -globin, and myoglobin are paralogs of each other.

The term "polynucleotide" denotes a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules.

Sizes of polynucleotides are expressed as base pairs (abbreviated "bp"), nucleotides ("nt"), or kilobases ("kb"). Where the context allows, the latter two terms may describe polynucleotides that are single-stranded or 5 double-stranded. When the term is applied to double-stranded molecules it is used to denote overall length and will be understood to be equivalent to the term "base pairs". It will be recognized by those skilled in the art that the two strands of a double-stranded polynucleotide 10 may differ slightly in length and that the ends thereof may be staggered as a result of enzymatic cleavage; thus all nucleotides within a double-stranded polynucleotide molecule may not be paired. Such unpaired ends will in general not exceed 20 nt in length.

15 "Probes and/or primers" as used herein can be RNA or DNA. DNA can be either cDNA or genomic DNA. Polynucleotide probes and primers are single or double-stranded DNA or RNA, generally synthetic oligonucleotides, but may be generated from cloned cDNA or genomic sequences 20 or its complements. Analytical probes will generally be at least 20 nucleotides in length, although somewhat shorter probes (14-17 nucleotides) can be used. PCR primers are at least 5 nucleotides in length, preferably 15 or more nt, more preferably 20-30 nt. Short 25 polynucleotides can be used when a small region of the gene is targeted for analysis. For gross analysis of genes, a polynucleotide probe may comprise an entire exon or more. Probes can be labeled to provide a detectable signal, such as with an enzyme, biotin, a radionuclide, 30 fluorophore, chemiluminescer, paramagnetic particle and the like, which are commercially available from many

The term "promoter" denotes a portion of a gene containing DNA sequences that provide for the binding of

RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule (i.e., a ligand) and mediates the effect of the ligand on the cell. Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction. Binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell. This interaction in turn leads to an alteration in the metabolism of the cell. Metabolic events that are linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids. Most nuclear receptors also exhibit a multi-domain structure, including an amino-terminal, transactivating domain, a DNA binding domain and a ligand binding domain. In general, receptors can be membrane bound, cytosolic or nuclear; monomeric (e.g., thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (e.g., PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor).

The term "secretory signal sequence" denotes a DNA sequence that encodes a polypeptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger peptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

The term "splice variant" is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA 5 molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a protein 10 encoded by a splice variant of an mRNA transcribed from a gene.

Molecular weights and lengths of polymers determined by imprecise analytical methods (e.g., gel electrophoresis) will be understood to be approximate 15 values. When such a value is expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to $\pm 10\%$.

The present invention is based in part upon the discovery of a novel DNA sequence that encodes a 20 polypeptide characterized by homology to mouse ventral prostate spermine-binding protein (SEQ ID NO: 3). See, for example, Mills et al., Nucleic Acids Res. 15: 7709-24, 1987. Possibly more significant is the homology of zsig32 polypeptide to *Rattus norvegicus* common salivary protein 25 (U00964_1; SEQ ID NO: 4), as discussed in Girard et al., J. Biol. Chem., 268(35): 26592-601, 1993, and murine common salivary protein 1 (S76879_1; SEQ ID NO: 5).

In addition, the zsig32 polypeptides of the present invention preferably incorporate one potential N-glycosylation site at amino acid 167 (Asn) of SEQ ID NO: 30 2. Zsig32 polypeptides of the present invention also

contain one or more putative phosphorylation sites. Such a site is located at amino acid 107 (Tyr) of SEQ ID NO: 2. Such putative sites of phosphorylation may indicate that

zsig32, like spermine-binding protein, is regulated by cAMP-independent protein kinases. See, for example, Goueli et al., Biochem. J. (England) 230(2): 293-302, 1985. In addition, the zsig32 polypeptides of the present invention preferably incorporate three potential casein kinase II phosphorylation sites at amino acids 35 (Ser), 36 (Tyr) and 107 (Tyr) of SEQ ID NO: 2. Such sites may indicate that zsig32 may be regulated by casein kinase II. Preferably, three potential N-myristoylation sites are located at amino acids 46, 72 and 120 of SEQ ID NO: 2. These sites are not 100% conserved in the aligned common salivary proteins.

Zsig32 polypeptides also preferably incorporate an adhesion motif (amino acid residues 63-65 of SEQ ID NO: 2). According to three-dimensional analysis of polypeptide structure, the adhesion motif appears to be exposed. Thus, zsig32 polypeptides and fragments thereof, incorporating such an adhesion motif, appear to be useful in the study of adhesion, as is more fully described herein. Also useful in this regard are fusion proteins containing zsig32 polypeptide or an exposed adhesion motif-containing fragment thereof.

The novel polypeptides of the present invention, designated zsig32 polypeptides, were initially identified by querying an EST database for secretory signal sequences characterized by an upstream methionine start site, a hydrophobic region of approximately 13 amino acids and a cleavage site (SEQ ID NO: 6, wherein cleavage occurs between the alanine and glycine amino acid residues) in an effort to select for secreted proteins. Polypeptides corresponding to ESTs meeting those search criteria were compared to known sequences to identify secreted proteins having homology to known ligands. A single EST sequence was discovered and predicted to be related to a secreted spermine-binding protein found in rat ventral prostate (SPBP). See, for example, Mills et al., Nucleic Acids

Res. 15: 7709-24, 1987. Homology was also discovered between zsig32 polypeptide and *Rattus norvegicus* common salivary protein 1 (U00964_1), as discussed in Girard et al., J. Biol. Chem., 268(35): 26592-601, 1993, and a 5 murine common salivary protein 1 (S76879_1).

The full sequence of the zsig32 polypeptide was obtained from a single clone believed to contain it, wherein the clone was obtained from a parotid gland tissue library. Other libraries that might also be searched for 10 such clones include submandibular gland, salivary gland, prostate, trachea, colon, stomach, prostate tumor, lung, thyroid, tongue tumor and the like.

Analysis of the tissue distribution of the mRNA corresponding to this novel DNA by Northern blot analysis 15 using a synthetic probe (SEQ ID NO: 7) showed that expression was highest in trachea, lower in prostate, and apparent but further decreased in colon and stomach. A tissue distribution analysis by Dot blot showed extremely high expression in salivary gland, with significantly less 20 expression in trachea and apparent but further decreased expression in prostate. In evaluating these results it is important to note that the Northern blot analysis did not include the salivary gland. A single transcript size of approximately 1 kb was observed.

25 The nucleotide sequence of the N-terminal EST is described in SEQ ID NO: 1, and its deduced amino acid sequence is described in SEQ ID NO: 2. Analysis of the DNA encoding a zsig32 polypeptide (SEQ ID NO: 1) revealed an open reading frame encoding either 178 (Met at amino 30 acid number 1 of SEQ ID NO: 2 is the start site) or 172 amino acids (Met at amino acid number 7 of SEQ ID NO: 2 is

start site; or 16 amino acid residues (residue 7 to residue 22 of SEQ ID NO: 2; Met at position 7 is the start

site) and a mature polypeptide of 156 amino acids (residue 23 to residue 178 of SEQ ID NO: 2). Those skilled in the art will recognize that predicted secretory signal sequence domain boundaries are approximations based on 5 primary sequence content, and may vary slightly; however, such estimates are generally accurate to within \pm 4 amino acid residues. Therefore the present invention also includes the polypeptides having amino acid sequences comprising amino acid residues 20-178 of SEQ ID NO:2, 10 residues 21-178 of SEQ ID NO:2, residues 22-178 of SEQ ID NO:2, residues 23-178 of SEQ ID NO:2, residues 24-178 of SEQ ID NO:2, residues 25-178, residues 26-178 of SEQ ID NO:2 and residues 27-178 of SEQ ID NO:2 as well as the polynucleotides encoding them.

15 An alignment was prepared including zsig32 polypeptide, mouse ventral prostate spermine-binding protein SPBP_MOUSE (SEQ ID NO: 3; Met at position 7) rat common salivary protein 1 (U00964_1; SEQ ID NO: 4; Met at positions 1 and 7), and murine common salivary protein 1 20 (S76879_1; SEQ ID NO: 5; Met at positions 1 and 7). That alignment, as shown in the Figure, revealed a block of high percent identity in the signal sequence and a block of significant percent identity in the mature protein corresponding to the region of SEQ ID NO: 2 from amino 25 acid residue 7 (Met), corresponding to aligned residue 7, to amino acid residue 217 (Glu), corresponding to aligned residue 167. When compared, the most conserved sequences are in the signal sequence. Also, the two common salivary proteins and zsig32 share a six amino acid presequence 30 (residues 1-6 of SEQ ID NOS. 4, 5 and 6). In addition, the C-terminal tail of SPBP_MOUSE appears to be longer than that of zsig32 and to be characterized by high asparagine/aspartic acid concentration. The C-terminal tail of SPBP_MOUSE is believed to participate in spermine 35 binding. In contrast, the C-terminal tails of the salivary proteins are shorter than that of zsig32.

Neither zsig32 nor the salivary proteins incorporate a region of high asparagine/aspartic acid concentration, potentially indicating alternative regulation or specificity.

5 Within the region of significant identity in the mature polypeptides, the following percent identity figures are observed for the deduced amino acid sequence of zsig32 polypeptide (SEQ ID NO: 2), SPBP_MOUSE (SEQ ID NO: 3), rat common salivary protein 1, U00964_1 (SEQ ID NO: 4), and murine common salivary protein 1, S76879_1 (SEQ ID NO: 5).

| | Zsig32 | U00964_1 | S76879_1 | SPBP_MOUSE |
|------------|--------|----------|----------|------------|
| Zsig32 | 100 | 31 | 29 | 29 |
| U00964_1 | 31 | 100 | 44 | 29 |
| S76879_1 | 29 | 44 | 100 | 28 |
| SPBP_MOUSE | 29 | 29 | 28 | 100 |

15 Highly conserved amino acids can be used as a tool to identify zsig32 polypeptides or other proteins characterized by salivary gland function indication or modulation, spermine or polyamine binding capability, cAMP-independent protein kinase- or androgen-regulation susceptibility or the like. For instance, reverse 20 transcription-polymerase chain reaction (RT-PCR) can be used to amplify sequences encoding a conserved motif from RNA obtained from a variety of tissue sources. In particular, the following primers are useful for this purpose.

25 1) Amino acids 14-19 of SEQ ID NO: 2
(corresponding to nucleotides 40-57 of SEQ ID NO: 1 nucleotides 40-57 of SEQ ID NO: 2)

corresponding to nucleotides 81-108 of SEQ ID

NO: 1, nucleotides 97-108 of SEQ ID NO:20 and their complements);

3) Amino acids 154-159 of SEQ ID NO: 2 (corresponding to nucleotides 460-477 of SEQ ID NO: 1, nucleotides 460-477 of SEQ ID NO:20 and their complements); and

5 4) Amino acids 109-114 of SEQ ID NO: 2 (corresponding to nucleotides 325-342 of SEQ ID NO: 1, nucleotides 325-342 of SEQ ID NO:20, and their complements).

10

The activity of polypeptides identified by such probes or of polypeptides encoded by polynucleotides identified by such probes can be determined by methods that are known in the art as generally described herein.

15 Oligonucleotide probes based on the polynucleotide sequence of SEQ ID NO:1 can be used to localize the zsig32 gene to a particular chromosome. Radiation hybrid mapping is a somatic cell genetic technique developed for constructing high-resolution, contiguous maps of mammalian chromosomes (Cox et al., Science 250:245-50, 1990). Partial or full knowledge of a gene's sequence allows one to design PCR primers suitable for use with chromosomal radiation hybrid mapping panels. Radiation hybrid mapping panels are commercially available

20 which cover the entire human genome, such as the Stanford G3 RH Panel and the GeneBridge 4 RH Panel (Research Genetics, Inc., Huntsville, AL). These panels enable rapid, PCR-based chromosomal localizations and ordering of genes, sequence-tagged sites (STSs), and other

25 nonpolymorphic and polymorphic markers within a region of interest. This includes establishing directly proportional physical distances between newly discovered genes of interest and previously mapped markers. The precise knowledge of a gene's position can be useful for a

30 number of purposes, including: 1) determining if a sequence is part of an existing contig and obtaining

35

additional surrounding genetic sequences in various forms, such as YACs, BACs or cDNA clones; 2) providing a possible candidate gene for an inheritable disease which shows linkage to the same chromosomal region; and 3) 5 cross-referencing model organisms, such as mouse, which may aid in determining what function a particular gene might have.

Sequence tagged sites (STSs) can also be used independently for chromosomal localization. An STS is a 10 DNA sequence that is unique in the human genome and can be used as a reference point for a particular chromosome or region of a chromosome. An STS is defined by a pair of oligonucleotide primers that are used in a polymerase chain reaction to specifically detect this site in the 15 presence of all other genomic sequences. Since STSs are based solely on DNA sequence they can be completely described within an electronic database, for example, Database of Sequence Tagged Sites (dbSTS), GenBank, (National Center for Biological Information, National 20 Institutes of Health, Bethesda, MD <http://www.ncbi.nlm.nih.gov>), and can be searched with a gene sequence of interest for the mapping data contained within these short genomic landmark STS sequences.

The results of chromosome mapping experiments, 25 as more fully described in Example 3 hereof, showed that the zsig32 gene maps 7.47 cR from the top of the human chromosome 16 linkage group on the WICGR radiation hybrid map. Relative to the centromere, its nearest proximal marker was WI-7742 and its nearest distal marker was WI- 30 3061. The use of surrounding markers positioned the zsig32 gene in the 16p13.3 region on the integrated LDB

that encode the zsig32 polypeptides disclosed herein. Those skilled in the art will readily recognize that, in

view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. SEQ ID NO:20 is a degenerate DNA sequence that encompasses all DNAs that encode the zsig32 polypeptide of 5 SEQ ID NO:2. Those skilled in the art will recognize that the degenerate sequence of SEQ ID NO:20 also provides all RNA sequences encoding SEQ ID NO:2 by substituting U for T. Thus, zsig32 polypeptide-encoding polynucleotides comprising nucleotide 61 to nucleotide 534 of SEQ ID 10 NO:20, nucleotide 22 to nucleotide 534 of SEQ ID NO:20 and nucleotide 1 to nucleotide 534 of SEQ ID NO:20 and their RNA equivalents are contemplated by the present invention. Table 1 sets forth the one-letter codes used within SEQ ID NO:20 to denote degenerate nucleotide positions. 15 "Resolutions" are the nucleotides denoted by a code letter. "Complement" indicates the code for the complementary nucleotide(s). For example, the code Y denotes either C or T, and its complement R denotes A or G, A being complementary to T, and G being complementary 20 to C.

TABLE 1

| Nucleotide | Resolution | Nucleotide | Complement |
|------------|------------|------------|------------|
| A | A | T | T |
| C | C | G | G |
| G | G | C | C |
| T | T | A | A |
| R | A G | Y | C T |
| Y | C T | R | A G |
| M | A C | K | G T |
| K | G T | M | A C |
| S | C G | S | C G |
| W | A T | W | A T |
| H | A C T | D | A G T |
| B | C G T | V | A C G |
| V | A C G | B | C G T |
| D | A G T | H | A C T |
| N | A C G T | N | A C G T |

The degenerate codons used in SEQ ID NO:20,
5 encompassing all possible codons for a given amino acid,
are set forth in Table 2.

TABLE 2

| Amino Acid | Letter Code | One | Codons | Degenerate Codon |
|------------|-------------|-----|---------------------|------------------|
| Cys | C | TGC | TGT | TGY |
| Ser | S | AGC | AGT TCA TCC TCG TCT | WSN |
| Thr | T | ACA | ACC ACG ACT | ACN |
| Pro | P | CCA | CCC CCG CCT | CCN |
| Ala | A | GCA | GCC GCG GCT | GCN |
| Gly | G | GGA | GGC GGG GGT | GGN |
| Asn | N | AAC | AAT | AAY |
| Asp | D | GAC | GAT | GAY |
| Glu | E | GAA | GAG | GAR |
| Gln | Q | CAA | CAG | CAR |
| His | H | CAC | CAT | CAY |
| Arg | R | AGA | AGG CGA CGC CGG CGT | MGN |
| Lys | K | AAA | AAG | AAR |
| Met | M | ATG | | ATG |
| Ile | I | ATA | ATC ATT | ATH |
| Leu | L | CTA | CTC CTG CTT TTA TTG | YTN |
| Val | V | GTA | GTC GTG GTT | GTN |
| Phe | F | TTC | TTT | TTY |
| Tyr | Y | TAC | TAT | TAY |
| Trp | W | TGG | | TGG |
| Ter | . | TAA | TAG TGA | TRR |
| Asn Asp | B | | | RAY |
| Glu Gln | Z | | | SAR |
| Any | X | | | NNN |

One of ordinary skill in the art will appreciate that some ambiguity is introduced in determining a degenerate codon, representative of all possible codons encoding each amino acid. For example, the degenerate 5 codon for serine (WSN) can, in some circumstances, encode arginine (AGR), and the degenerate codon for arginine (MGN) can, in some circumstances, encode serine (AGY). A similar relationship exists between codons encoding phenylalanine and leucine. Thus, some polynucleotides 10 encompassed by the degenerate sequence may encode variant amino acid sequences, but one of ordinary skill in the art can easily identify such variant sequences by reference to the amino acid sequence of SEQ ID NO:2. Variant sequences can be readily tested for functionality as described 15 herein.

One of ordinary skill in the art will also appreciate that different species can exhibit "preferential codon usage." In general, see, Grantham, et al., Nuc. Acids Res. 8:1893-912, 1980; Haas, et al. Curr. 20 Biol. 6:315-24, 1996; Wain-Hobson, et al., Gene 13:355-64, 1981; Grosjean and Fiers, Gene 18:199-209, 1982; Holm, Nuc. Acids Res. 14:3075-87, 1986; Ikemura, J. Mol. Biol. 158:573-97, 1982. As used herein, the term "preferential codon usage" or "preferential codons" is a term of art 25 referring to protein translation codons that are most frequently used in cells of a certain species, thus favoring one or a few representatives of the possible codons encoding each amino acid (See Table 2). For example, the amino acid threonine (Thr) may be encoded by 30 ACA, ACC, ACG, or ACT, but in mammalian cells ACC is the most commonly used codon; in other species, for example, ~~the same codon may be used in different amounts in different species~~

polynucleotides of the present invention by a variety of methods known in the art. Introduction of preferential 10 codon usage into a polynucleotide of the present invention can be achieved by a variety of methods known in the art. For example, a polynucleotide of the present invention can be introduced into a host cell, and the host cell can be cultured to express the polynucleotide. The polynucleotide can then be harvested and purified. The polynucleotide can be used in a variety of applications, such as gene therapy, gene cloning, and gene expression analysis.

enhance production of the protein by making protein translation more efficient within a particular cell type or species. Therefore, the degenerate codon sequence disclosed in SEQ ID NO:20 serves as a template for 5 optimizing expression of polynucleotides in various cell types and species commonly used in the art and disclosed herein. Sequences containing preferential codons can be tested and optimized for expression in various species, and tested for functionality as disclosed herein.

10 Based upon homology to spermine binding protein, zsig32 polypeptides may be used in the study of cAMP-independent protein kinases or androgens. Androgens mediate cAMP-independent protein kinase levels, which in turn mediate phosphorylation of zsig32 polypeptides. In 15 an embodiment of the present invention, immobilized zsig32 polypeptides are incubated with γ -³²P-ATP and a cAMP-independent protein kinase, and incorporation of ³²P into zsig32 polypeptides is monitored by known techniques. Alternatively, cells that express or are engineered to 20 express zsig32 polypeptides and that endogenously express one or more cAMP-independent protein kinase are incubated with androgens and γ -³²P-ATP. The cells are then lysed and incorporation of ³²P into zsig32 polypeptides is monitored by known techniques. In addition, spermine 25 binding protein mRNA has been suggested as a marker for specific androgen activity, as described in Labrie et al., Endocrinology 124(6): 2745-54, 1989. Zsig32 polypeptide mRNA may also be useful for that purpose.

As shown in the Figure and described herein, 30 zsig32 polypeptides also exhibit homology to common salivary protein 1 isolated from rat and mouse. Moreover, zsig32 polypeptides are characterized by a limited tissue distribution. While the Northern blot and Dot blot analyses are not in complete agreement, a high level of 35 expression of zsig32 polypeptide was observed in the salivary gland. Consequently, another aspect of the

present invention involves the detection of zsig32 polypeptides in the serum or tissue biopsy of a patient undergoing evaluation for salivary gland function or dysfunction. Such zsig32 polypeptides can be detected 5 using immunoassay techniques and antibodies capable of recognizing zsig32 polypeptide epitopes.

More specifically, the present invention contemplates methods for detecting zsig32 polypeptide comprising:

10 exposing a solution possibly containing zsig32 polypeptide to an antibody attached to a solid support, wherein said antibody binds to a first epitope of a zsig32 polypeptide;

15 washing said immobilized antibody-polypeptide to remove unbound contaminants;

exposing the immobilized antibody-polypeptide to a second antibody directed to a second epitope of a zsig32 polypeptide, wherein the second antibody is associated with a detectable label; and

20 detecting the detectable label. Serum or biopsy zsig32 polypeptide concentration (relative to normal serum or tissue concentration) may be indicative of dysfunction of the salivary gland. Salivary gland dysfunctions include digestive dysfunction, wound healing dysfunction, 25 inadequate saliva production or composition, mucosal integrity breakdown, failure or diminished anti-microbial function. Detection of zsig32 polypeptide at relatively high levels in the trachea may indicate that such polypeptides may serve as a marker of lung dysfunction.

30 Examples of conditions associated with salivary gland or lung dysfunction include salivary gland carcinoma, 35 and 40

45 addition, zsig32 polypeptides are expressed in the prostate, albeit at a lower level than in the salivary

regulated and shares other properties with salivary glands. Consequently, dysfunction thereof, such as prostate adenocarcinoma or the like, may also be detected using zsig32 polypeptides.

5 Also, the salivary glands synthesize and secrete a number of proteins having diverse biological functions. Such proteins facilitate lubrication of the oral cavity (e.g., mucins and proline-rich proteins), remineralization (e.g., statherin and ionic proline-rich proteins),
10 digestion (e.g., amylase, lipase and proteases), provide anti-microbial (e.g., proline-rich proteins, lysozyme, histatins and lactoperoxidase) and mucosal integrity maintenance (e.g., mucins) capabilities. In addition, saliva is a rich source of growth factors synthesized by
15 the salivary glands. For example, saliva is known to contain epidermal growth factor (EGF), nerve growth factor (NGF), transforming growth factor-alpha (TGF- α), transforming growth factor-beta (TGF- β), insulin, insulin-like growth factors I and II (IGF-I and IGF-II) and
20 fibroblast growth factor (FGF). See, for example, Zelles et al., J. Dental. Res. 74: 1826-32, 1995. Synthesis of growth factors by the salivary gland is believed to be androgen-dependent and to be necessary for the health of the oral cavity and gastrointestinal tract.

25 Thus, zsig32 polypeptides, agonists or antagonists thereof may be therapeutically useful for aiding digestion. To verify the presence of this capability in zsig32 polypeptides, agonists or antagonists of the present invention, such zsig32 polypeptides, agonists or antagonists are evaluated with respect to
30 their ability to break down starch according to procedures known in the art. If desired, zsig32 polypeptide performance in this regard can be compared to digestive enzymes, such as amylase, lipase, proteases and the like.
35 In addition, zsig32 polypeptides or agonists or antagonists thereof may be evaluated in combination with

one or more digestive enzymes to identify synergistic effects.

Also, zsig32 polypeptides, agonists or antagonists thereof may be therapeutically useful for 5 promoting wound healing. To verify the presence of this capability in zsig32 polypeptides, agonists or antagonists of the present invention, such zsig32 polypeptides, agonists or antagonists are evaluated with respect to their ability to facilitate wound healing according to 10 procedures known in the art. If desired, zsig32 polypeptide performance in this regard can be compared to growth factors, such as EGF, NGF, TGF- α , TGF- β , insulin, IGF-I, IGF-II, fibroblast growth factor (FGF) and the like. In addition, zsig32 polypeptides or agonists or 15 antagonists thereof may be evaluated in combination with one or more growth factors to identify synergistic effects.

In addition, zsig32 polypeptides, agonists or antagonists thereof may be therapeutically useful for 20 anti-microbial applications. To verify the presence of this capability in zsig32 polypeptides, agonists or antagonists of the present invention, such zsig32 polypeptides, agonists or antagonists are evaluated with respect to their anti-microbial properties according to 25 procedures known in the art. See, for example, Barsum et al., Eur. Respir. J. 8: 709-14, 1995; Sandovsky-Losica et al., J. Med. Vet. Mycol. (England) 28: 279-87, 1990; Mehentee et al., J. Gen. Microbiol. (England) 135 (Pt. 8): 2181-8, 1989; Segal and Savage, J. Med. Vet. Mycol. 24: 30 477-9, 1986 and the like. If desired, zsig32 polypeptide performance in this regard can be compared to proteins

antagonists thereof may be evaluated in combination with

one or more anti-microbial agents to identify synergistic effects.

Anti-microbial protective agents may be directly acting or indirectly acting. Such agents operating via membrane association or pore forming mechanisms of action directly attach to the offending microbe. Anti-microbial agents can also act via an enzymatic mechanism, breaking down microbial protective substances or the cell wall/membrane thereof. Anti-microbial agents, capable of inhibiting microorganism proliferation or action or of disrupting microorganism integrity by either mechanism set forth herein, are useful in methods for preventing contamination in cell culture by microbes susceptible to that anti-microbial activity. Such techniques involve culturing cells in the presence of an effective amount of said zsig32 polypeptide or an agonist or antagonist thereof.

Also, zsig32 polypeptides or agonists thereof may be used as cell culture reagents in *in vitro* studies of exogenous microorganism infection, such as bacterial, viral or fungal infection. Such moieties may also be used in *in vivo* animal models of infection. Also, the microorganism-adherence properties of zsig32 polypeptides or agonists thereof can be studied under a variety of conditions in binding assays and the like.

Moreover, zsig32 polypeptides, agonists or antagonists thereof may be therapeutically useful for mucosal integrity maintenance. To verify the presence of this capability in zsig32 polypeptides, agonists or antagonists of the present invention, such zsig32 polypeptides, agonists or antagonists are evaluated with respect to their mucosal integrity maintenance according to procedures known in the art. See, for example, Zahm et al., Eur. Respir. J. 8: 381-6, 1995, which describes methods for measuring viscoelastic properties and surface properties of mucous as well as for evaluating mucous

transport by cough and by ciliary activity. If desired, zsig32 polypeptide performance in this regard can be compared to mucins or the like. In addition, zsig32 polypeptides or agonists or antagonists thereof may be 5 evaluated in combination with mucins to identify synergistic effects.

In addition, a 37 amino acid peptide has been discovered which is secreted in saliva and believed to cause vasodilation of cranial blood vessels. Such 10 vasodilation leads to migraine attacks. This peptide is believed to be secreted in response to clenching of the teeth, which is also often associated with migraine attacks. Thus, a further aspect of the present invention involves the determination of the vasodilatory effects of 15 zsig32 polypeptides and agonists and antagonists thereof. Such determination may be made using known assay techniques. Compounds capable of down-modulating vasodilation of blood vessels may be useful in the prevention or treatment of migraine attacks.

20 Polynucleotides encoding zsig32 polypeptides are useful within gene therapy applications where it is desired to increase or inhibit zsig32 activity. If a mammal has a mutated or absent zsig32 gene, the zsig32 gene can be introduced into the cells of the mammal. In 25 one embodiment, a gene encoding a zsig32 polypeptide is introduced *in vivo* in a viral vector. Such vectors include an attenuated or defective DNA virus, such as, but not limited to, herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, 30 adeno-associated virus (AAV), and the like. Defective viruses, which entirely or almost entirely lack viral

localized area, without concern that the vector can infect other cells. Examples of particular vectors include, but

are not limited to, a defective herpes simplex virus 1 (HSV1) vector (Kaplitt et al., Molec. Cell. Neurosci. 2:320-30, 1991); an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al., J. Clin. Invest. 90:626-30, 1992; and a defective adeno-associated virus vector (Samulski et al., J. Virol. 61:3096-101, 1987; Samulski et al., J. Virol. 63:3822-8, 1989).

In another embodiment, a zsig32 gene can be introduced in a retroviral vector, e.g., as described in Anderson et al., U.S. Patent NO: 5,399,346; Mann et al. Cell 33:153, 1983; Temin et al., U.S. Patent NO: 4,650,764; Temin et al., U.S. Patent NO: 4,980,289; Markowitz et al., J. Virol. 62:1120, 1988; Temin et al., U.S. Patent NO: 5,124,263; International Patent Publication NO: WO 95/07358, published March 16, 1995 by Dougherty et al.; and Kuo et al., Blood 82:845, 1993. Alternatively, the vector can be introduced by lipofection *in vivo* using liposomes. Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7, 1987; Mackey et al., Proc. Natl. Acad. Sci. USA 85:8027-31, 1988). The use of lipofection to introduce exogenous genes into specific organs *in vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. More particularly, directing transfection to particular cells represents one area of benefit. For instance, directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides (e.g., hormones or neurotransmitters), proteins such as antibodies, or non-peptide molecules can be coupled to liposomes chemically.

It is possible to remove the target cells from the body; to introduce the vector as a naked DNA plasmid; and then to re-implant the transformed cells into the body. Naked DNA vectors for gene therapy can be 5 introduced into the desired host cells by methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun or use of a DNA vector transporter. See, e.g., Wu et al., J. 10 Biol. Chem. 267:963-7, 1992; Wu et al., J. Biol. Chem. 263:14621-4, 1988.

Antisense methodology can be used to inhibit zsig32 gene transcription, such as to inhibit cell proliferation *in vivo*. Polynucleotides that are 15 complementary to a segment of a zsig32-encoding polynucleotide (e.g., a polynucleotide as set forth in SEQ ID NO:1) are designed to bind to zsig32-encoding mRNA and to inhibit translation of such mRNA. Such antisense polynucleotides are used to inhibit expression of zsig32 20 polypeptide-encoding genes in cell culture or in a subject.

Transgenic mice, engineered to express the zsig32 gene, and mice that exhibit a complete absence of zsig32 gene function, referred to as "knockout mice" 25 (Snouwaert et al., Science 257:1083, 1992), may also be generated (Lowell et al., Nature 366:740-42, 1993). These mice may be employed to study the zsig32 gene and the protein encoded thereby in an *in vivo* system.

The present invention also provides reagents for 30 use in diagnostic applications. For example, the zsig32 gene, a probe comprising zsig32 DNA or RNA, or a

detectable chromosomal aberrations at the zsig32 gene locus include, but are not limited to, 35 chromosomal deletions, insertions, rearrangements, and

deletions, restriction site changes and rearrangements. These aberrations can occur within the coding sequence, within introns, or within flanking sequences, including upstream promoter and regulatory regions, and may be 5 manifested as physical alterations within a coding sequence or changes in gene expression level.

In general, these diagnostic methods comprise the steps of (a) obtaining a genetic sample from a patient; (b) incubating the genetic sample with a 10 polynucleotide probe or primer as disclosed above, under conditions wherein the polynucleotide will hybridize to complementary polynucleotide sequence, to produce a first reaction product; and (iii) comparing the first reaction product to a control reaction product. A difference 15 between the first reaction product and the control reaction product is indicative of a genetic abnormality in the patient. Genetic samples for use within the present invention include genomic DNA, cDNA, and RNA. The polynucleotide probe or primer can be RNA or DNA, and will comprise a portion of SEQ ID NO:1, the complement of SEQ 20 ID NO:1, or an RNA equivalent thereof. Suitable assay methods in this regard include molecular genetic techniques known to those in the art, such as restriction fragment length polymorphism (RFLP) analysis, short tandem 25 repeat (STR) analysis employing PCR techniques, ligation chain reaction (Barany, PCR Methods and Applications 1:5-16, 1991), ribonuclease protection assays, and other genetic linkage analysis techniques known in the art (Sambrook et al., ibid.; Ausubel et. al., ibid.; Marian, 30 Chest 108:255-65, 1995). Ribonuclease protection assays (see, e.g., Ausubel et al., ibid., ch. 4) comprise the hybridization of an RNA probe to a patient RNA sample, after which the reaction product (RNA-RNA hybrid) is exposed to RNase. Hybridized regions of the RNA are 35 protected from digestion. Within PCR assays, a patient's genetic sample is incubated with a pair of polynucleotide

primers, and the region between the primers is amplified and recovered. Changes in size or amount of recovered product are indicative of mutations in the patient. Another PCR-based technique that can be employed is single strand conformational polymorphism (SSCP) analysis (Hayashi, PCR Methods and Applications 1:34-8, 1991).

Within another aspect of the present invention there is provided a pharmaceutical composition comprising purified zsig32 polypeptide in combination with a pharmaceutically acceptable vehicle. Such pharmaceutical compositions may be administered to prevent or treat salivary gland dysfunction. Such prevention or treatment may be directed to digestive dysfunction, such as a deficiency in starch breakdown capability or efficiency, wound healing dysfunction, inadequate saliva production or composition or mucosal integrity breakdown. Zsig32 polypeptides may also have an anti-microbial function, most likely stemming from an exposed adhesion motif as discussed herein. Also, expression of zsig32 polypeptide at a relatively high level in trachea may indicate a role for zsig32 polypeptides in prevention or treatment of destructive lung disease. Examples of pathological conditions, characterized by one or more of the aforementioned criteria, include xerostomia, sarcoidosis, dental caries, osteomyelitis, oral candidiasis, buccal mucosa infections, chronic inflammation (Sjogren's syndrome), mumps, chronic bronchitis, adult respiratory distress syndrome (ARDS), sudden infant death syndrome (SIDS), salivary gland carcinoma, pneumocystic carinii (particularly as associated with AIDS patients), cystic fibrosis, emphysema and the like.

Evaluation of zsig32 polypeptide development 1.

art. For example, bronchoalveolar lavage may be employed in the assessment of destructive lung diseases, such as

pulmonary emphysema, chronic bronchitis, cystic fibrosis, ARDS and the like. See, for example, Luisetti et al., Respiration 59(suppl. 1): 24-7, 1992. Salivary gland, lacrimal gland and labial salivary gland biopses may be 5 employed in the evaluation of xerostomia. See, for example, Matsumoto et al., J. Clin. Invest. 97(8): 1969-77, 1996. This calcium channel dependent condition has also been evaluated using fura-2 assays of intracellular calcium ion concentration, as described in Seagrave et 10 al., Archs. Oral Biol. 41(5): 425-30, 1996. Alymphoplasia (aly) mice are a useful animal model for systemic Sjogren's syndrome, an autoimmune disease characterized by lymphocytic infiltration into the lachrymal and salivary glands, leading to symptomatic dry eyes and mouth. See, 15 for example, Furukawa et al., Brit. J. Rheum. 35: 1223-30, 1996.

The present invention provides methods for identifying agonists or antagonists of the zsig32 polypeptides disclosed herein, which agonists or 20 antagonists may have valuable therapeutic properties as discussed further herein. Within one embodiment, there is provided a method of identifying zsig32 polypeptide agonists, comprising providing cells responsive to a zsig32 polypeptide as disclosed herein, culturing the 25 cells in the presence of a test compound and comparing the cellular response with the cell cultured in the presence of the zsig32 polypeptide, and selecting the test compounds for which the cellular response is of the same type. Agonists are therefore useful to mimic or augment 30 the function of zsig32 polypeptides.

Within another embodiment, there is provided a method of identifying antagonists of zsig32 polypeptide, comprising providing cells responsive to a zsig32 polypeptide, culturing a first portion of the cells in the 35 presence of zsig32 polypeptide, culturing a second portion of the cells in the presence of the zsig32 polypeptide and

a test compound, and detecting a decrease in a cellular response of the second portion of the cells as compared to the first portion of the cells. Antagonists are therefore useful to inhibit or diminish zsig32 polypeptide function.

5 Zsig32 polypeptides of the present invention exhibit an adhesion motif at amino acid residues 63-65 of SEQ ID NO: 2. According to three-dimensional analysis of polypeptide structure, the adhesion motif appears to be exposed. Thus, zsig32 polypeptides, fragments thereof 10 incorporating such an adhesion motif or fusion proteins incorporating zsig32 polypeptide or the adhesion motif-containing fragment thereof appear to be useful in the study of adhesion. Such study can be accomplished using a method of modulating adhesion of platelets, for example, 15 in cell culture, comprising incubating platelets in a culture medium comprising a zsig32 polypeptide as disclosed above in an amount sufficient to modulate adhesion. Assays for evaluation of platelet adhesion are known in the art.

20 Zsig32 polypeptides or antagonists or agonists thereof are expected be useful in circumstances where modulation of adhesion is desired. Such adhesion-modulating function may be used in *in vitro* experiments 25 designed to study adhesion, such as adhesion of microorganisms to cells, tissue or mucous. Enhancers and inhibitors of adhesion also have potential as therapeutics for conditions requiring such modulation. For example, enhanced tumor cell-tumor cell adhesion in a primary solid tumor does not favor metastasis thereof. Also, diminished 30 tumor cell-endothelial cell adhesion also does not favor metastasis formation at a site distant from the primary

A still further aspect of the invention provides useful research reagents and methods for evaluating salivary gland function. Zsig32 polypeptides, agonists or antagonists thereof can be admixed with test saliva or one or more proteins contained in saliva to provide culture conditions under which salivary gland function can be studied. For example, admixture of zsig32 polypeptides, agonists or antagonists thereof can be combined with one or more growth factors to provide a culture medium in which the wound healing properties of saliva can be studied.

Within preferred embodiments of the invention the isolated polynucleotides will hybridize to similar sized regions of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, or SEQ ID NO: 11, the other specific probes referred to herein, or a sequence complementary thereto, under stringent conditions. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typical stringent conditions are those in which the salt concentration is less than about 0.02 M at pH 7 and the temperature is at least about 60°C.

As previously noted, the isolated zsig32 polynucleotides of the present invention include DNA and RNA. Methods for isolating DNA and RNA are well known in the art. It is generally preferred to isolate RNA from salivary gland tissues, although DNA can also be prepared using RNA from other tissues or isolated as genomic DNA. Total RNA can be prepared using guanidine HCl extraction followed by isolation by centrifugation in a CsCl gradient (Chirgwin et al., Biochemistry 18:52-94, 1979). Poly (A)⁺ RNA is prepared from total RNA using the method of Aviv and Leder (Proc. Natl. Acad. Sci. USA 69:1408-12, 1972).

Complementary DNA (cDNA) is prepared from poly(A)⁺ RNA using known methods. Polynucleotides encoding zsig32 polypeptides are then identified and isolated by, for example, hybridization or PCR.

5 The present invention further provides counterpart polypeptides and polynucleotides from other species (orthologs). These species include, but are not limited to mammalian, avian, amphibian, reptile, fish, insect and other vertebrate and invertebrate species. Of 10 particular interest are zsig32 polypeptides from other mammalian species, including murine, rat, porcine, ovine, bovine, canine, feline, equine and other primate proteins. Species homologs of the human proteins can be cloned using 15 information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses the protein. Suitable sources of mRNA can be identified by probing Northern blots with probes designed from the 20 sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line. A zsig32-encoding cDNA can then be isolated by a variety of methods, such as by probing with a complete or partial 25 human cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction, or PCR (Mullis, U.S. Patent 4,683,202), using primers designed from the sequences disclosed herein. Within an additional method, the cDNA library can be used to transform or 30 transfect host cells, and expression of the cDNA of interest can be detected with an antibody to an epitope of a zsig32 polypeptide. Similar techniques can also be

sequences disclosed in SEQ ID NO:1 and SEQ ID NO:2 represent a single allele of the human zsig32 gene and

polypeptide, and that allelic variation and alternative splicing are expected to occur. Allelic variants can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. Allelic 5 variants of the DNA sequence shown in SEQ ID NO: 1, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of SEQ ID NO:2. cDNAs generated 10 from alternatively spliced mRNAs, which retain the properties of the zsig32 polypeptide are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. Allelic variants and splice variants of these sequences can be cloned by 15 probing cDNA or genomic libraries from different individuals or tissues according to standard procedures known in the art.

The present invention also provides isolated zsig32 polypeptides that are substantially homologous to 20 the polypeptides of SEQ ID NO:2 and their orthologs. The term "substantially homologous" is used herein to denote polypeptides having 50%, preferably 60%, more preferably at least 80%, sequence identity to the sequences shown in SEQ ID NO:2 or their orthologs. Such polypeptides will 25 more preferably be at least 90% identical, and most preferably 95% or more identical to SEQ ID NO:2 or its orthologs. Percent sequence identity is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48: 603-16, 1986 and Henikoff and 30 Henikoff, Proc. Natl. Acad. Sci. USA 89:10915-9, 1992. Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "blosum 62" scoring matrix of Henikoff and Henikoff (ibid.) as shown in Table

3 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as:

$$5 \frac{\text{Total number of identical matches}}{\text{[length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences]}} \times 100$$

10

Table 3

| | A | R | N | D | C | Q | E | G | H | I | L | K | M | F | P | S | T | W | Y | V |
|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|---|---|
| A | 4 | | | | | | | | | | | | | | | | | | | |
| R | -1 | 5 | | | | | | | | | | | | | | | | | | |
| N | -2 | 0 | 6 | | | | | | | | | | | | | | | | | |
| D | -2 | -2 | 1 | 6 | | | | | | | | | | | | | | | | |
| C | 0 | -3 | -3 | -3 | 9 | | | | | | | | | | | | | | | |
| Q | -1 | 1 | 0 | 0 | -3 | 5 | | | | | | | | | | | | | | |
| E | -1 | 0 | 0 | 2 | -4 | 2 | 5 | | | | | | | | | | | | | |
| G | 0 | -2 | 0 | -1 | -3 | -2 | -2 | 6 | | | | | | | | | | | | |
| H | -2 | 0 | 1 | -1 | -3 | 0 | 0 | -2 | 8 | | | | | | | | | | | |
| I | -1 | -3 | -3 | -1 | -3 | -3 | -3 | -4 | -3 | 4 | | | | | | | | | | |
| L | -1 | -2 | -3 | -4 | -1 | -2 | -3 | -4 | -3 | 2 | 4 | | | | | | | | | |
| K | -1 | 2 | 0 | -1 | -3 | 1 | 1 | -2 | -1 | -3 | -2 | 5 | | | | | | | | |
| M | -1 | -1 | -2 | -3 | -1 | 0 | -2 | -3 | -2 | 1 | 2 | 1 | | | | | | | | |
| F | -2 | -3 | -3 | -2 | -3 | -3 | -3 | -1 | 0 | 0 | -3 | 0 | 6 | | | | | | | |
| P | -1 | -2 | -1 | -3 | -1 | -1 | -2 | -2 | -3 | -3 | -1 | -2 | -4 | 7 | | | | | | |
| S | 1 | -1 | 1 | 0 | -1 | 0 | 0 | -1 | -2 | -2 | 0 | -1 | -2 | -1 | 4 | | | | | |
| T | 0 | -1 | 0 | -1 | -1 | -1 | -1 | -2 | -1 | -1 | -1 | -1 | -2 | -1 | 1 | 5 | | | | |
| W | -3 | -4 | -4 | -2 | -2 | -3 | -2 | -2 | -3 | -2 | -3 | -1 | 1 | -4 | -3 | -2 | 11 | | | |
| Y | -2 | -2 | -3 | -2 | -1 | -2 | -3 | 2 | -1 | -1 | -2 | -1 | 3 | -3 | -2 | -2 | 2 | 7 | | |
| V | 0 | -3 | -3 | -1 | -2 | -2 | -3 | -3 | 3 | 1 | -2 | 1 | -1 | -2 | -2 | 0 | -3 | -1 | 4 | |

Sequence identity of polynucleotide molecules is determined by similar methods using a ratio as disclosed above.

Substantially homologous proteins and 5 polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see Table 4) and other substitutions that do not significantly affect the folding 10 or activity of the protein or polypeptide; small deletions, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or an affinity tag. 15 Polypeptides comprising affinity tags can further comprise a proteolytic cleavage site between the zsig32 polypeptide and the affinity tag. Preferred such sites include thrombin cleavage sites and factor Xa cleavage sites.

Table 4

Conservative amino acid substitutions

| | | |
|----|--------------|---------------|
| | Basic: | arginine |
| | | lysine |
| 5 | | histidine |
| | Acidic: | glutamic acid |
| | | aspartic acid |
| | Polar: | glutamine |
| | | asparagine |
| 10 | Hydrophobic: | leucine |
| | | isoleucine |
| | | valine |
| | Aromatic: | phenylalanine |
| | | tryptophan |
| 15 | | tyrosine |
| | Small: | glycine |
| | | alanine |
| | | serine |
| | | threonine |
| 20 | | methionine |

The present invention further provides a variety of other polypeptide fusions [and related multimeric proteins comprising one or more polypeptide fusions]. For example, a zsig32 polypeptide can be prepared as a fusion to a dimerizing protein as disclosed in U.S. Patents Nos. 5,155,027 and 5,567,584. Preferred dimerizing proteins in this regard include immunoglobulin constant region domains. Immunoglobulin-zsig32 polypeptide fusions can be expressed in genetically engineered cells [to produce a variety of multimeric zsig32 analogs]. Auxiliary domains can be fused to zsig32 polypeptides to target them to specific cells, tissues, or macromolecules. For example, a zsig32 polypeptide or protein could be targeted to a predetermined cell type by fusing a zsig32 polypeptide to a ligand that specifically binds to a receptor on the

surface of the target cell. In this way, polypeptides and proteins can be targeted for therapeutic or diagnostic purposes. A zsig32 polypeptide can be fused to two or more moieties, such as an affinity tag for purification and a targeting domain. Polypeptide fusions can also comprise one or more cleavage sites, particularly between domains. See, Tuan et al., Connect. Tiss. Res. 34:1-9, 1996.

The proteins of the present invention can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-hydroxyproline, *N*-methyl-glycine, *allo*-threonine, methylthreonine, hydroxyethyl-cysteine, hydroxyethylhomocysteine, nitroglutamine, homo-glutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethyl-proline, *tert*-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenyl-alanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is carried out in a cell-free system comprising an *E. coli* S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson et al., J. Am. Chem. Soc. 113:2722,

method, translation is carried out in *Xenopus* oocytes by microinjection of mutated mRNA and chemically

aminoacylated suppressor tRNAs (Turcatti et al., J. Biol. Chem. 271:19991-8, 1996). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced (e.g., phenylalanine) and in the 5 presence of the desired non-naturally occurring amino acid(s) (e.g., 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. See, Koide 10 et al., Biochem. 33:7470-6, 1994. Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions 15 (Wynn and Richards, Protein Sci. 2:395-403, 1993).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for zsig32 amino acid 20 residues.

Essential amino acids in the zsig32 polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham 25 and Wells, Science 244: 1081-5, 1989). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity (e.g., androgen regulation, anti-microbial activity, adhesion 30 modulation or the like) to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton et al., J. Biol. Chem. 271:4699-708, 1996. Sites of ligand-receptor interaction can also be determined by physical analysis of structure, as 35 determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or

photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos et al., Science 255:306-12, 1992; Smith et al., J. Mol. Biol. 224:899-904, 1992; Wlodaver et al., FEBS Lett. 309:59-64, 1992. The identities of essential amino acids can also be inferred from analysis of homologies with related proteins.

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (Science 241:53-7, 1988) or Bowie and Sauer (Proc. Natl. Acad. Sci. USA 86:2152-6, 1989). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (e.g., Lowman et al., Biochem. 30:10832-7, 1991; Ladner et al., U.S. Patent NO: 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis (Derbyshire et al., Gene 46:145, 1986; Ner et al., DNA 7:127, 1988).

Variants of the disclosed zsig32 DNA and polypeptide sequences can be generated through DNA shuffling as disclosed by Stemmer, Nature 370:389-91, 1994, Stemmer, Proc. Natl. Acad. Sci. USA 91:10747-51, 1994 and WIPO Publication WO 97/20078. Briefly, variant DNAs are generated by *in vitro* homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using

the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid "evolution" of

sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

Mutagenesis methods as disclosed above can be combined with high-throughput, automated screening methods 5 to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode active polypeptides (e.g., those susceptible to androgen regulation, capable of anti-microbial action or adhesion modulation or the like) can be recovered from the host 10 cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

15 Using the methods discussed herein, one of ordinary skill in the art can identify and/or prepare a variety of polypeptides that are substantially homologous to residues 23 (Gly) to 178 (Arg) of SEQ ID NO: 2 or allelic variants thereof and retain the one or more 20 properties of the wild-type protein. Such polypeptides may include additional amino acids, such as affinity tags or the like. Such polypeptides may also include additional polypeptide segments as generally discussed herein.

25 The polypeptides of the present invention, including full-length proteins, fragments thereof and fusion proteins, can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or 30 transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured cells of multicellular organisms, are preferred. Techniques for manipulating cloned DNA molecules and 35 introducing exogenous DNA into a variety of host cells are disclosed by Sambrook et al., Molecular Cloning: A

Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, and Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley and Sons, Inc., NY, 1987.

5 In general, a DNA sequence encoding a zsig32
polypeptide of the present invention is operably linked to
other genetic elements required for its expression,
generally including a transcription promoter and
terminator within an expression vector. The vector will
10 also commonly contain one or more selectable markers and
one or more origins of replication, although those skilled
in the art will recognize that within certain systems
selectable markers may be provided on separate vectors,
and replication of the exogenous DNA may be provided by
15 integration into the host cell genome. Selection of
promoters, terminators, selectable markers, vectors and
other elements is a matter of routine design within the
level of ordinary skill in the art. Many such elements
are described in the literature and are available through
20 commercial suppliers.

To direct a zsig32 polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The secretory signal sequence may be that of the zsig32 polypeptide, or may be derived from another secreted protein (e.g., t-PA) or synthesized de novo. The secretory signal sequence is joined to the zsig32-encoding DNA sequence in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although

5,143,830). Conversely, the signal sequence portion of the zsig32 polypeptide (amino acids 1-22 or 7-22 of SEQ ID

NO: 2) may be employed to direct the secretion of an alternative protein by analogous methods.

Cultured mammalian cells are also preferred hosts within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler et al., Cell 14:725, 1978; Corsaro and Pearson, Somatic Cell Genetics 7:603, 1981; Graham and Van der Eb, Virology 52:456, 1973), electroporation (Neumann et al., EMBO J. 1:841-845, 1982), DEAE-dextran mediated transfection (Ausubel et al., eds., Current Protocols in Molecular Biology, John Wiley and Sons, Inc., NY, 1987), liposome-mediated transfection (Hawley-Nelson et al., Focus 15:73, 1993; Ciccarone et al., Focus 15:80, 1993), and viral vectors (Miller and Rosman, BioTechniques 7:980-90, 1989; Wang and Finer, Nat. Med. 2:714-16, 1996). The production of recombinant polypeptides in cultured mammalian cells is disclosed, for example, by Levinson et al., U.S. Patent NO: 4,713,339; Hagen et al., U.S. Patent NO: 4,784,950; Palmiter et al., U.S. Patent NO: 4,579,821; and Ringold, U.S. Patent NO: 4,656,134. Suitable cultured mammalian cells include the COS-1 (ATCC NO: CRL 1650), COS-7 (ATCC NO: CRL 1651), BHK 570 (ATCC NO: CRL 10314), 293 (ATCC NO: CRL 1573; Graham et al., J. Gen. Virol. 36:59-72, 1977) and Chinese hamster ovary (e.g. CHO-K1; ATCC NO: CCL 61) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Rockville, Maryland. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, e.g., U.S. Patent NO: 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patent Nos. 4,579,821 and 4,601,978) and the adenovirus major late promoter.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been

inserted. Such cells are commonly referred to as "transfectants". Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as 5 "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems may also be used to increase the expression level of the 10 gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high 15 levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g., hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can 20 also be used. Alternative markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by such means 25 as FACS sorting or magnetic bead separation technology.

Other higher eukaryotic cells can also be used as hosts, including insect cells, plant cells and avian cells. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by 30 Sinkar et al., J. Biosci. (Bangalore) 11:47-58, 1987. Transformation of insect cells and production of foreign

can be infected with recombinant baculovirus, commonly derived from *Autographa californica* nuclear polyhedrosis

virus (AcNPV). DNA encoding the zsig32 polypeptide is inserted into the baculoviral genome in place of the AcNPV polyhedrin gene coding sequence by one of two methods. The first is the traditional method of homologous DNA 5 recombination between wild-type AcNPV and a transfer vector containing the zsig32 flanked by AcNPV sequences. Suitable insect cells, e.g. SF9 cells, are infected with wild-type AcNPV and transfected with a transfer vector comprising a zsig32 polynucleotide operably linked to an 10 AcNPV polyhedrin gene promoter, terminator, and flanking sequences. See, King, and Possee, The Baculovirus Expression System: A Laboratory Guide, London, Chapman & Hall; O'Reilly et al., Baculovirus Expression Vectors: A Laboratory Manual, New York, Oxford University Press., 15 1994; and, Richardson, Ed., Baculovirus Expression Protocols. Methods in Molecular Biology, Totowa, NJ, Humana Press, 1995. Natural recombination within an insect cell will result in a recombinant baculovirus which contains zsig32 driven by the polyhedrin promoter. 20 Recombinant viral stocks are made by methods commonly used in the art.

The second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow (Luckow, et al., J. Virol. 67:4566-79, 1993). 25 This system is sold in the Bac-to-Bac kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, pFastBac1™ (Life Technologies) containing a Tn7 transposon to move the DNA encoding the zsig32 polypeptide into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." The pFastBac1™ transfer vector utilizes the AcNPV polyhedrin promoter to drive the expression of the gene of interest, in this case zsig32. However, pFastBac1™ can be modified to a considerable degree. The polyhedrin promoter can be 30 removed and substituted with the baculovirus basic protein promoter (also known as P_{cor}, p6.9 or MP promoter) which 35

is expressed earlier in the baculovirus infection, and has been shown to be advantageous for expressing secreted proteins. See, Hill-Perkins and Possee, J. Gen. Virol. 71:971-6, 1990; Bonning et al., J. Gen. Virol. 75:1551-6, 1994; and, Chazenbalk and Rapoport, J. Biol. Chem. 270:1543-9, 1995. In such transfer vector constructs, a short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed which replace the native zsig32 secretory signal sequences with secretory signal sequences derived from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin (Invitrogen, Carlsbad, CA), or baculovirus gp67 (PharMingen, San Diego, CA) can be used in constructs to replace the native zsig32 secretory signal sequence. In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed zsig32 polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer et al., ibid.). Using techniques known in the art, a transfer vector containing zsig32 is transformed into *E. coli*, and screened for bacmids which contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is isolated, using common techniques, and used to transfect *Spodoptera frugiperda* cells, e.g. Sf9 cells. Recombinant virus that expresses zsig32 is subsequently produced. Recombinant viral stocks are made by methods commonly used the art.

The recombinant virus is used to infect host cells, typically a cell line derived from the fall armyworm, *Spodoptera frugiperda*. See, in general, Glick

... . Another suitable cell line is the high five T cell line (Invitrogen) derived from *Trichoplusia ni* (U.S.

Patent #5,300,435). Commercially available serum-free media are used to grow and maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cell0405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the *T. ni* cells. The cells are grown up from an inoculation density of approximately $2-5 \times 10^5$ cells to a density of $1-2 \times 10^6$ cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3. The recombinant virus-infected cells typically produce the recombinant zsig32 polypeptide at 12-72 hours post-infection and secrete it with varying efficiency into the medium. The culture is usually harvested 48 hours post-infection. Centrifugation is used to separate the cells from the medium (supernatant). The supernatant containing the zsig32 polypeptide is filtered through micropore filters, usually 0.45 μm pore size. Procedures used are generally described in available laboratory manuals (King and Possee, *ibid.*; O'Reilly, et al., *ibid.*; Richardson, *ibid.*). Subsequent purification of the zsig32 polypeptide from the supernatant can be achieved using methods described herein.

Fungal cells, including yeast cells, and particularly cells of the genus *Saccharomyces*, can also be used within the present invention, such as for producing zsig32 polypeptide fragments or polypeptide fusions. Methods for transforming yeast cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent NO: 4,599,311; Kawasaki et al., U.S. Patent NO: 4,931,373; Brake, U.S. Patent NO: 4,870,008; Welch et al., U.S. Patent NO: 5,037,743; and Murray et al., U.S. Patent NO: 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a

particular nutrient (e.g., leucine). A preferred vector system for use in yeast is the POT1 vector system disclosed by Kawasaki et al. (U.S. Patent NO: 4,931,373), which allows transformed cells to be selected by growth in 5 glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent NO: 4,599,311; Kingsman et al., U.S. Patent NO: 4,615,974; and Bitter, U.S. Patent NO: 4,977,092) and alcohol 10 dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago 15 maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guillermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson et al., J. Gen. Microbiol. 132:3459-3465, 1986 and Cregg, U.S. Patent NO: 4,882,279. Aspergillus cells may be utilized according to the methods 20 of McKnight et al., U.S. Patent NO: 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino et al., U.S. Patent NO: 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent NO: 4,486,533.

25 The use of *Pichia methanolica* as host for the production of recombinant proteins is disclosed in WIPO Publications WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming *P. methanolica* will commonly be prepared as double-stranded, 30 circular plasmids, which are preferably linearized prior to transformation. For recombinant production, a

gene, such as a *P. methanolica* alcohol utilization gene (AUG1 or AUG2). Other useful promoters include those of

the dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To facilitate integration of the DNA into the host chromosome, it is preferred to have the entire expression segment of the plasmid flanked at both ends by host DNA sequences. A preferred selectable marker for use in *Pichia methanolica* is a *P. methanolica* *ADE2* gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase (AIRC; EC 4.1.1.21), which allows *ade2* host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, it is preferred to use host cells in which both methanol utilization genes (*AUG1* and *AUG2*) are deleted. For production of secreted proteins, host cells deficient in vacuolar protease genes (*PEP4* and *PRB1*) are preferred. Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of interest into *P. methanolica* cells. It is preferred to transform *P. methanolica* cells by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 3.75 kV/cm, and a time constant (τ) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

Prokaryotic host cells, including strains of the bacteria *Escherichia coli*, *Bacillus* and other genera are also useful host cells within the present invention. Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well known in the art (see, e.g., Sambrook et al., *ibid.*). When expressing a *zsig32* polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured polypeptide can then be refolded

and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, 5 the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for 10 denaturation and refolding.

Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of 15 suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium 20 will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell. *P. methanolica* cells are 25 cultured in a medium comprising adequate sources of carbon, nitrogen and trace nutrients at a temperature of about 25°C to 35°C. Liquid cultures are provided with sufficient aeration by conventional means, such as shaking of small flasks or sparging of fermentors. A preferred 30 culture medium for *P. methanolica* is YEPD (2% D-glucose, 2% Bacto™ Peptone (Difco Laboratories, Detroit, MI), 1%

chimeric zsig32 polypeptides) can be purified using fractionation and/or conventional purification methods and

media. Ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase 5 high performance liquid chromatography. Suitable chromatographic media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are preferred, with DEAE Fast-Flow Sepharose (Pharmacia, Piscataway, NJ) 10 being particularly preferred. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic 15 resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble 20 under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate 25 moieties. Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other 30 solid media are well known and widely used in the art, and are available from commercial suppliers. Methods for binding receptor polypeptides to support media are well known in the art. Selection of a particular method is a matter of routine design and is determined in part by the 35 properties of the chosen support. See, for example, Affinity Chromatography: Principles & Methods, Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988.

The polypeptides of the present invention can be isolated by exploitation of their structural properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins, including those comprising polyhistidine tags. Briefly, a gel is first charged with divalent metal ions to form a chelate (Sulkowski, Trends in Biochem. 3:1-7, 1985). Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography (Methods in Enzymol., Vol. 182, "Guide to Protein Purification", M. Deutscher, (ed.), Acad. Press, San Diego, 1990, pp.529-39). Within additional embodiments of the invention, a fusion of the polypeptide of interest and an affinity tag (e.g., maltose-binding protein, Glu-Glu, an immunoglobulin domain) may be constructed to facilitate purification.

Protein refolding (and optionally reoxidation) procedures may be advantageously used. It is preferred to purify the protein to >80% purity, more preferably to >90% purity, even more preferably >95%, and particularly preferred is a pharmaceutically pure state, that is greater than 99.9% pure with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. Preferably, a purified protein is substantially free of other proteins, particularly other proteins of animal origin.

Zsiga30 polypeptides or fragments thereof may

... glycosylated; pegylated or non-pegylated; and may or may not include an initial methionine amino acid residue.

A zsig32 polypeptide binding protein can also be used in purification applications. The binding protein is immobilized on a solid support, such as beads of agarose, cross-linked agarose, glass, cellulosic resins, silica-based resins, polystyrene, cross-linked polyacrylamide, or like materials that are stable under the conditions of use. Methods for linking polypeptides to solid supports are known in the art, and include amine chemistry, cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, and hydrazide activation. The resulting medium will generally be configured in the form of a column, and fluids containing ligand are passed through the column one or more times to allow ligand (e.g., zsig32 polypeptide) to bind to the receptor/binding protein. The ligand is then eluted using changes in salt concentration, chaotropic agents (guanidine HCl), or pH to disrupt ligand-receptor binding.

An assay system that uses a ligand-binding receptor (or an antibody, one member of a complement/anti-complement pair) or a binding fragment thereof, and a commercially available biosensor instrument (BIAcore™, Pharmacia Biosensor, Piscataway, NJ) may be advantageously employed. Such receptor, antibody, member of a complement/anti-complement pair or fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, J. Immunol. Methods 145:229-40, 1991 and Cunningham and Wells, J. Mol. Biol. 234:554-63, 1993. A receptor, antibody, member or fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell. If a ligand, epitope, or opposite member of the complement/anti-complement pair is present in the sample, it will bind to the immobilized receptor, antibody or member, respectively, causing a change in the

refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and 5 assessment of stoichiometry of binding.

Ligand-binding receptor polypeptides can also be used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity (see Scatchard, Ann. NY Acad. Sci. 51: 10 660-72, 1949) and calorimetric assays (Cunningham et al., Science 253:545-48, 1991; Cunningham et al., Science 245:821-25, 1991).

Zsig32 can be measured *in vitro* using cultured cells or *in vivo* by administering molecules of the claimed 15 invention to the appropriate animal model. For instance, zsig32 transfected (or co-transfected) expression host cells may be embedded in an alginate environment and injected (implanted) into recipient animals. Alginate-poly-L-lysine microencapsulation, permselective membrane 20 encapsulation and diffusion chambers have been described as a means to entrap transfected mammalian cells or primary mammalian cells. These types of non-immunogenic "encapsulations" or microenvironments permit the transfer of nutrients into the microenvironment, and also permit 25 the diffusion of proteins and other macromolecules secreted or released by the captured cells across the environmental barrier to the recipient animal. Most importantly, the capsules or microenvironments mask and shield the foreign, embedded cells from the recipient 30 animal's immune response. Such microenvironments can extend the life of the injected cells from a few hours or

10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400 405 410 415 420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495 500 505 510 515 520 525 530 535 540 545 550 555 560 565 570 575 580 585 590 595 600 605 610 615 620 625 630 635 640 645 650 655 660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 905 910 915 920 925 930 935 940 945 950 955 960 965 970 975 980 985 990 995 1000 1005 1010 1015 1020 1025 1030 1035 1040 1045 1050 1055 1060 1065 1070 1075 1080 1085 1090 1095 1100 1105 1110 1115 1120 1125 1130 1135 1140 1145 1150 1155 1160 1165 1170 1175 1180 1185 1190 1195 1200 1205 1210 1215 1220 1225 1230 1235 1240 1245 1250 1255 1260 1265 1270 1275 1280 1285 1290 1295 1300 1305 1310 1315 1320 1325 1330 1335 1340 1345 1350 1355 1360 1365 1370 1375 1380 1385 1390 1395 1400 1405 1410 1415 1420 1425 1430 1435 1440 1445 1450 1455 1460 1465 1470 1475 1480 1485 1490 1495 1500 1505 1510 1515 1520 1525 1530 1535 1540 1545 1550 1555 1560 1565 1570 1575 1580 1585 1590 1595 1600 1605 1610 1615 1620 1625 1630 1635 1640 1645 1650 1655 1660 1665 1670 1675 1680 1685 1690 1695 1700 1705 1710 1715 1720 1725 1730 1735 1740 1745 1750 1755 1760 1765 1770 1775 1780 1785 1790 1795 1800 1805 1810 1815 1820 1825 1830 1835 1840 1845 1850 1855 1860 1865 1870 1875 1880 1885 1890 1895 1900 1905 1910 1915 1920 1925 1930 1935 1940 1945 1950 1955 1960 1965 1970 1975 1980 1985 1990 1995 2000 2005 2010 2015 2020 2025 2030 2035 2040 2045 2050 2055 2060 2065 2070 2075 2080 2085 2090 2095 2100 2105 2110 2115 2120 2125 2130 2135 2140 2145 2150 2155 2160 2165 2170 2175 2180 2185 2190 2195 2200 2205 2210 2215 2220 2225 2230 2235 2240 2245 2250 2255 2260 2265 2270 2275 2280 2285 2290 2295 2300 2305 2310 2315 2320 2325 2330 2335 2340 2345 2350 2355 2360 2365 2370 2375 2380 2385 2390 2395 2400 2405 2410 2415 2420 2425 2430 2435 2440 2445 2450 2455 2460 2465 2470 2475 2480 2485 2490 2495 2500 2505 2510 2515 2520 2525 2530 2535 2540 2545 2550 2555 2560 2565 2570 2575 2580 2585 2590 2595 2600 2605 2610 2615 2620 2625 2630 2635 2640 2645 2650 2655 2660 2665 2670 2675 2680 2685 2690 2695 2700 2705 2710 2715 2720 2725 2730 2735 2740 2745 2750 2755 2760 2765 2770 2775 2780 2785 2790 2795 2800 2805 2810 2815 2820 2825 2830 2835 2840 2845 2850 2855 2860 2865 2870 2875 2880 2885 2890 2895 2900 2905 2910 2915 2920 2925 2930 2935 2940 2945 2950 2955 2960 2965 2970 2975 2980 2985 2990 2995 3000 3005 3010 3015 3020 3025 3030 3035 3040 3045 3050 3055 3060 3065 3070 3075 3080 3085 3090 3095 3100 3105 3110 3115 3120 3125 3130 3135 3140 3145 3150 3155 3160 3165 3170 3175 3180 3185 3190 3195 3200 3205 3210 3215 3220 3225 3230 3235 3240 3245 3250 3255 3260 3265 3270 3275 3280 3285 3290 3295 3300 3305 3310 3315 3320 3325 3330 3335 3340 3345 3350 3355 3360 3365 3370 3375 3380 3385 3390 3395 3400 3405 3410 3415 3420 3425 3430 3435 3440 3445 3450 3455 3460 3465 3470 3475 3480 3485 3490 3495 3500 3505 3510 3515 3520 3525 3530 3535 3540 3545 3550 3555 3560 3565 3570 3575 3580 3585 3590 3595 3600 3605 3610 3615 3620 3625 3630 3635 3640 3645 3650 3655 3660 3665 3670 3675 3680 3685 3690 3695 3700 3705 3710 3715 3720 3725 3730 3735 3740 3745 3750 3755 3760 3765 3770 3775 3780 3785 3790 3795 3800 3805 3810 3815 3820 3825 3830 3835 3840 3845 3850 3855 3860 3865 3870 3875 3880 3885 3890 3895 3900 3905 3910 3915 3920 3925 3930 3935 3940 3945 3950 3955 3960 3965 3970 3975 3980 3985 3990 3995 4000 4005 4010 4015 4020 4025 4030 4035 4040 4045 4050 4055 4060 4065 4070 4075 4080 4085 4090 4095 4100 4105 4110 4115 4120 4125 4130 4135 4140 4145 4150 4155 4160 4165 4170 4175 4180 4185 4190 4195 4200 4205 4210 4215 4220 4225 4230 4235 4240 4245 4250 4255 4260 4265 4270 4275 4280 4285 4290 4295 4300 4305 4310 4315 4320 4325 4330 4335 4340 4345 4350 4355 4360 4365 4370 4375 4380 4385 4390 4395 4400 4405 4410 4415 4420 4425 4430 4435 4440 4445 4450 4455 4460 4465 4470 4475 4480 4485 4490 4495 4500 4505 4510 4515 4520 4525 4530 4535 4540 4545 4550 4555 4560 4565 4570 4575 4580 4585 4590 4595 4600 4605 4610 4615 4620 4625 4630 4635 4640 4645 4650 4655 4660 4665 4670 4675 4680 4685 4690 4695 4700 4705 4710 4715 4720 4725 4730 4735 4740 4745 4750 4755 4760 4765 4770 4775 4780 4785 4790 4795 4800 4805 4810 4815 4820 4825 4830 4835 4840 4845 4850 4855 4860 4865 4870 4875 4880 4885 4890 4895 4900 4905 4910 4915 4920 4925 4930 4935 4940 4945 4950 4955 4960 4965 4970 4975 4980 4985 4990 4995 5000 5005 5010 5015 5020 5025 5030 5035 5040 5045 5050 5055 5060 5065 5070 5075 5080 5085 5090 5095 5100 5105 5110 5115 5120 5125 5130 5135 5140 5145 5150 5155 5160 5165 5170 5175 5180 5185 5190 5195 5200 5205 5210 5215 5220 5225 5230 5235 5240 5245 5250 5255 5260 5265 5270 5275 5280 5285 5290 5295 5300 5305 5310 5315 5320 5325 5330 5335 5340 5345 5350 5355 5360 5365 5370 5375 5380 5385 5390 5395 5400 5405 5410 5415 5420 5425 5430 5435 5440 5445 5450 5455 5460 5465 5470 5475 5480 5485 5490 5495 5500 5505 5510 5515 5520 5525 5530 5535 5540 5545 5550 5555 5560 5565 5570 5575 5580 5585 5590 5595 5600 5605 5610 5615 5620 5625 5630 5635 5640 5645 5650 5655 5660 5665 5670 5675 5680 5685 5690 5695 5700 5705 5710 5715 5720 5725 5730 5735 5740 5745 5750 5755 5760 5765 5770 5775 5780 5785 5790 5795 5800 5805 5810 5815 5820 5825 5830 5835 5840 5845 5850 5855 5860 5865 5870 5875 5880 5885 5890 5895 5900 5905 5910 5915 5920 5925 5930 5935 5940 5945 5950 5955 5960 5965 5970 5975 5980 5985 5990 5995 6000 6005 6010 6015 6020 6025 6030 6035 6040 6045 6050 6055 6060 6065 6070 6075 6080 6085 6090 6095 6100 6105 6110 6115 6120 6125 6130 6135 6140 6145 6150 6155 6160 6165 6170 6175 6180 6185 6190 6195 6200 6205 6210 6215 6220 6225 6230 6235 6240 6245 6250 6255 6260 6265 6270 6275 6280 6285 6290 6295 6300 6305 6310 6315 6320 6325 6330 6335 6340 6345 6350 6355 6360 6365 6370 6375 6380 6385 6390 6395 6400 6405 6410 6415 6420 6425 6430 6435 6440 6445 6450 6455 6460 6465 6470 6475 6480 6485 6490 6495 6500 6505 6510 6515 6520 6525 6530 6535 6540 6545 6550 6555 6560 6565 6570 6575 6580 6585 6590 6595 6600 6605 6610 6615 6620 6625 6630 6635 6640 6645 6650 6655 6660 6665 6670 6675 6680 6685 6690 6695 6700 6705 6710 6715 6720 6725 6730 6735 6740 6745 6750 6755 6760 6765 6770 6775 6780 6785 6790 6795 6800 6805 6810 6815 6820 6825 6830 6835 6840 6845 6850 6855 6860 6865 6870 6875 6880 6885 6890 6895 6900 6905 6910 6915 6920 6925 6930 6935 6940 6945 6950 6955 6960 6965 6970 6975 6980 6985 6990 6995 7000 7005 7010 7015 7020 7025 7030 7035 7040 7045 7050 7055 7060 7065 7070 7075 7080 7085 7090 7095 7100 7105 7110 7115 7120 7125 7130 7135 7140 7145 7150 7155 7160 7165 7170 7175 7180 7185 7190 7195 7200 7205 7210 7215 7220 7225 7230 7235 7240 7245 7250 7255 7260 7265 7270 7275 7280 7285 7290 7295 7300 7305 7310 7315 7320 7325 7330 7335 7340 7345 7350 7355 7360 7365 7370 7375 7380 7385 7390 7395 7400 7405 7410 7415 7420 7425 7430 7435 7440 7445 7450 7455 7460 7465 7470 7475 7480 7485 7490 7495 7500 7505 7510 7515 7520 7525 7530 7535 7540 7545 7550 7555 7560 7565 7570 7575 7580 7585 7590 7595 7600 7605 7610 7615 7620 7625 7630 7635 7640 7645 7650 7655 7660 7665 7670 7675 7680 7685 7690 7695 7700 7705 7710 7715 7720 7725 7730 7735 7740 7745 7750 7755 7760 7765 7770 7775 7780 7785 7790 7795 7800 7805 7810 7815 7820 7825 7830 7835 7840 7845 7850 7855 7860 7865 7870 7875 7880 7885 7890 7895 7900 7905 7910 7915 7920 7925 7930 7935 7940 7945 7950 7955 7960 7965 7970 7975 7980 7985 7990 7995 8000 8005 8010 8015 8020 8025 8030 8035 8040 8045 8050 8055 8060 8065 8070 8075 8080 8085 8090 8095 8100 8105 8110 8115 8120 8125 8130 8135 8140 8145 8150 8155 8160 8165 8170 8175 8180 8185 8190 8195 8200 8205 8210 8215 8220 8225 8230 8235 8240 8245 8250 8255 8260 8265 8270 8275 8280 8285 8290 8295 8300 8305 8310 8315 8320 8325 8330 8335 8340 8345 8350 8355 8360 8365 8370 8375 8380 8385 8390 8395 8400 8405 8410 8415 8420 8425 8430 8435 8440 8445 8450 8455 8460 8465 8470 8475 8480 8485 8490 8495 8500 8505 8510 8515 8520 8525 8530 8535 8540 8545 8550 8555 8560 8565 8570 8575 8580 8585 8590 8595 8600 8605 8610 8615 8620 8625 8630 8635 8640 8645 8650 8655 8660 8665 8670 8675 8680 8685 8690 8695 8700 8705 8710 8715 8720 8725 8730 8735 8740 8745 8750 8755 8760 8765 8770 8775 8780 8785 8790 8795 8800 8805 8810 8815 8820 8825 8830 8835 8840 8845 8850 8855 8860 8865 8870 8875 8880 8885 8890 8895 8900 8905 8910 8915 8920 8925 8930 8935 8940 8945 8950 8955 8960 8965 8970 8975 8980 8985 8990 8995 9000 9005 9010 9015 9020 9025 9030 9035 9040 9045 9050 9055 9060 9065 9070 9075 9080 9085 9090 9095 9100 9105 9110 9115 9120 9125 9130 9135 9140 9145 9150 9155 9160 9165 9170 9175 9180 9185 9190 9195 9200 9205 9210 9215 9220 9225 9230 9235 9240 9245 9250 9255 9260 9265 9270 9275 9280 9285 9290 9295 9300 9305 9310 9315 9320 9325 9330 9335 9340 9345 9350 9355 9360 9365 9370 9375 9380 9385 9390 9395 9400 9405 9410 9415 9420 9425 9430 9435 9440 9445 9450 9455 9460 9465 9470 9475 9480 9485 9490 9495 9500 9505 9510 9515 9520 9525 9530 9535 9540 9545 9550 9555 9560 9565 9570 9575 9580 9585 9590 9595 9600 9605 9610 9615 9620 9625 9630 9635 9640 9645 9650 9655 9660 9665 9670 9675 9680 9685 9690 9695 9700 9705 9710 9715 9720 9725 9730 9735 9740 9745 9750 9755 9760 9765 9770 9775 9780 9785 9790 9795 9800 9805 9810 9815 9820 9825 9830 9835 9840 9845 9850 9855 9860 9865 9870 9875 9880 9885 9890 9895 9900 9905 9910 9915 9920 9925 9930 9935 9940 9945 9950 9955 9960 9965 9970 9975 9980 9985 9990 9995 9999

are relatively strong and durable, both *in vitro* and, based on data obtained using the threads, *in vivo*. The alginic threads are easily manipulable and the methodology is scalable for preparation of numerous 5 threads. In an exemplary procedure, 3% alginic is prepared in sterile H₂O, and sterile filtered. Just prior to preparation of alginic threads, the alginic solution is again filtered. An approximately 50% cell suspension (containing about 5 x 10⁵ to about 5 x 10⁷ cells/ml) is 10 mixed with the 3% alginic solution. One ml of the alginic/cell suspension is extruded into a 100 mM sterile filtered CaCl₂ solution over a time period of ~15 min, forming a "thread". The extruded thread is then transferred into a solution of 50 mM CaCl₂, and then into 15 a solution of 25 mM CaCl₂. The thread is then rinsed with deionized water before coating the thread by incubating in a 0.01% solution of poly-L-lysine. Finally, the thread is rinsed with Lactated Ringer's Solution and drawn from solution into a syringe barrel (without needle attached). 20 A large bore needle is then attached to the syringe, and the thread is intraperitoneally injected into a recipient in a minimal volume of the Lactated Ringer's Solution.

An alternative *in vivo* approach for assaying 25 proteins of the present invention involves viral delivery systems. Exemplary viruses for this purpose include adenovirus, herpesvirus, vaccinia virus and adeno-associated virus (AAV). Adenovirus, a double-stranded DNA virus, is currently the best studied gene transfer vector for delivery of heterologous nucleic acid (for a review, 30 see T.C. Becker et al., Meth. Cell Biol. 43:161-89, 1994; and Douglas and Curiel, Science & Medicine 4:44-53, 1997). The adenovirus system offers several advantages: adenovirus can (i) accommodate relatively large DNA 35 inserts; (ii) be grown to high-titer; (iii) infect a broad range of mammalian cell types; and (iv) be used with a large number of available vectors containing different

promoters. Also, because adenoviruses are stable in the bloodstream, they can be administered by intravenous injection.

By deleting portions of the adenovirus genome, 5 larger inserts (up to 7 kb) of heterologous DNA can be accommodated. These inserts can be incorporated into the viral DNA by direct ligation or by homologous recombination with a co-transfected plasmid. In an exemplary system, the essential E1 gene has been deleted 10 from the viral vector, and the virus will not replicate unless the E1 gene is provided by the host cell (the human 293 cell line is exemplary). When intravenously administered to intact animals, adenovirus primarily targets the liver. If the adenoviral delivery system has 15 an E1 gene deletion, the virus cannot replicate in the host cells. However, the host's tissue (e.g., liver) will express and process (and, if a secretory signal sequence is present, secrete) the heterologous protein. Secreted proteins will enter the circulation in the highly 20 vascularized liver, and effects on the infected animal can be determined.

The adenovirus system can also be used for protein production *in vitro*. By culturing adenovirus-infected non-293 cells under conditions where the cells 25 are not rapidly dividing, the cells can produce proteins for extended periods of time. For instance, BHK cells are grown to confluence in cell factories, then exposed to the adenoviral vector encoding the secreted protein of interest. The cells are then grown under serum-free 30 conditions, which allows infected cells to survive for several weeks without significant cell division.

¹³ Garnier et al., Cytotechnol. 15:145-55, 1994). With either protocol, an expressed, secreted heterologous

protein can be repeatedly isolated from the cell culture supernatant. Within the infected 293S cell production protocol, non-secreted proteins may also be effectively obtained. Zsig32 polypeptides can also be used to prepare 5 antibodies that specifically bind to zsig32 epitopes, peptides or polypeptides. Antibodies generated from this immune response can be isolated and purified as described herein. Methods for preparing and isolating polyclonal and monoclonal antibodies are well known in the art. See, 10 for example, Current Protocols in Immunology, Cooligan, et al. (eds.), National Institutes of Health, John Wiley and Sons, Inc., 1995; Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY, 1989; and Hurrell, J. G. R., Ed., Monoclonal Hybridoma 15 Antibodies: Techniques and Applications, CRC Press, Inc., Boca Raton, FL, 1982.

As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from inoculating a variety of warm-blooded animals such as 20 horses, cows, goats, sheep, dogs, chickens, rabbits, mice, hamsters, guinea pigs and rats as well as transgenic animals such as transgenic sheep, cows, goats or pigs. Antibodies may also be expressed in yeast and fungi in modified forms as well as in mammalian and insect cells. 25 The zsig32 polypeptide or a fragment thereof serves as an antigen (immunogen) to inoculate an animal or elicit an immune response. Suitable antigens would include the zsig32 polypeptide encoded by SEQ ID NO:2 from amino acid residue 21-175 of SEQ ID NO:2, or a contiguous 9-175 amino 30 acid residue fragment thereof. The immunogenicity of a zsig32 polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as 35 fusions of zsig32 or a portion thereof with an immunoglobulin polypeptide or with maltose binding

protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like", such portion may be advantageously joined or linked to a macromolecular carrier (such as 5 keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

As used herein, the term "antibodies" includes polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding 10 fragments, such as $F(ab')_2$ and Fab proteolytic fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain 15 antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. Non-human antibodies may be humanized by grafting non-human CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains 20 (optionally "cloaking" them with a human-like surface by replacement of exposed residues, wherein the result is a "veeneered" antibody). In some instances, humanized antibodies may retain non-human residues within the human variable region framework domains to enhance proper 25 binding characteristics. Through humanizing antibodies, biological half-life may be increased, and the potential for adverse immune reactions upon administration to humans is reduced. Human antibodies can also be made in mice 30 having a humanized humoral immune system (Mendez et al., Nat. Genet. 14:146- 56, 1997).

Alternative techniques for generating or 30 selecting antibodies useful herein include *in vitro* exposure of lymphocytes to zsig32 protein or peptide, and

methods discussed herein, in particular domain shuffling, can be used to generate and mature antibodies.

The antibodies of the current invention, or fragments thereof, can be used to direct molecules to a specific target. For example, as T-bodies, chimeric receptors combining antibody recognition with T cell effector function, (Eshhar et al., Springer Semin Immunopathol. 18:199-209, 1996; Eshhar, Cancer Immunol. Immunother. 45:131-6, 1997). Intrabodies, engineered single-chain antibodies expressed inside the cell and having high affinity and specificity for intracellular targets. Such molecules have use in gene therapy and treatment of infectious diseases (Marasco, Immunotechnology 1:1-19, 1995; Marasco et al., Gene Ther. 4:11-5, 1997; Rondon and Marasco, Annu. Rev. Microbiol. 51:257-83, 1997 and Mhashilkar et al., J. Virol. 71:6486-94, 1997). Diabodies, bispecific non-covalent dimers of scFv antibodies useful for immunodiagnosis and therapeutically. In addition they can be constructed in bacteria (Holliger et al., Proc. Natl. Acad. Sci. USA 90:6444-48, 1993).

Antibodies herein specifically bind if they bind to a zsig32 polypeptide, peptide or epitope with a binding affinity (K_a) of 10^6 M^{-1} or greater, preferably 10^7 M^{-1} or greater, more preferably 10^8 M^{-1} or greater, and most preferably 10^9 M^{-1} or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard, ibid.).

Genes encoding polypeptides having potential zsig32 polypeptide binding domains, "binding proteins", can be obtained by screening random or directed peptide libraries displayed on phage (phage display) or on bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. Alternatively, constrained phage display libraries can also be produced. These peptide display

libraries can be used to screen for peptides which interact with a known target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such peptide display libraries are known in the art (Ladner et al., US Patent NO. 5,223,409; Ladner et al., US Patent NO. 4,946,778; Ladner et al., US Patent NO. 5,403,484 and Ladner et al., US Patent NO. 5,571,698) and peptide display libraries and kits for screening such libraries are available commercially, for instance from Clontech (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA) and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Peptide display libraries can be screened using the zsig32 sequences disclosed herein to identify proteins which bind to zsig32. These "binding proteins" which interact with zsig32 polypeptides can be used essentially like an antibody, for tagging cells; for isolating homolog polypeptides by affinity purification; directly or indirectly conjugated to drugs, toxins, radionuclides and the like. These binding proteins can also be used in analytical methods such as for screening expression libraries and neutralizing activity. The binding proteins can also be used for diagnostic assays for determining circulating levels of polypeptides; for detecting or quantitating soluble polypeptides as marker of underlying pathology or disease. To increase the half-life of these binding proteins, they can be conjugated. Their biological properties may be modified by dimerizing or multimerizing for use as agonists or antagonists.

proteins or peptides. Exemplary assays are described in detail in Antibodies: A Laboratory Manual, Harlow and Lane

(Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include: concurrent immunoelectrophoresis, radioimmunoassay, radioimmuno-precipitation, enzyme-linked immunosorbent assay (ELISA), 5 dot blot or Western blot assay, inhibition or competition assay, and sandwich assay. In addition, antibodies can be screened for binding to wild-type versus mutant zsig32 protein or polypeptide.

Antibodies and binding proteins to zsig32 may be 10 used for tagging cells that express zsig32; for isolating zsig32 by affinity purification; for diagnostic assays for determining circulating levels of zsig32 polypeptides; for detecting or quantitating soluble zsig32 as marker of underlying pathology or disease; in analytical methods 15 employing FACS; for screening expression libraries; for generating anti-idiotypic antibodies; and as neutralizing antibodies or as antagonists to block zsig32 polypeptide adhesion modulating or anti-microbial or like activity *in vitro* and *in vivo*. Suitable direct tags or labels include 20 radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like; indirect tags or labels may feature use of biotin-avidin or other complement/anti-complement pairs as intermediates. Antibodies herein may 25 also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic or therapeutic applications. Moreover, antibodies to zsig32 or fragments thereof may be 30 used *in vitro* to detect denatured zsig32 or fragments thereof in assays, for example, Western Blots or other assays known in the art.

In another embodiment, polypeptide-toxin fusion proteins or antibody-toxin fusion proteins can be used for targeted cell or tissue inhibition or ablation (for 35 instance, to treat cancer cells or tissues). Alternatively, if the polypeptide has multiple functional

domains (i.e., an activation domain or a ligand binding domain, plus a targeting domain), a fusion protein including only the targeting domain may be suitable for directing a detectable molecule, a cytotoxic molecule or a 5 complementary molecule to a cell or tissue type of interest. In instances where the domain only fusion protein includes a complementary molecule, the anti-complementary molecule can be conjugated to a detectable or cytotoxic molecule. Such domain-complementary molecule 10 fusion proteins thus represent a generic targeting vehicle for cell/tissue-specific delivery of generic anti-complementary-detectable/cytotoxic molecule conjugates. The bioactive polypeptide or antibody conjugates described herein can be delivered intravenously, intraarterially, 15 intraductally with DMSO, intramuscularly, subcutaneously, intraperitoneally, also by transdermal methods, by electro-transfer, orally or via inhalant.

Molecules of the present invention can be used to identify and isolate receptors involved in salivary 20 gland function or saliva composition. For example, proteins and peptides of the present invention can be immobilized on a column and membrane preparations run over the column (Immobilized Affinity Ligand Techniques, Hermanson et al., eds., Academic Press, San Diego, CA, 25 1992, pp.195-202). Proteins and peptides can also be radiolabeled (Methods in Enzymol., vol. 182, "Guide to Protein Purification", M. Deutscher, ed., Acad. Press, San Diego, 1990, 721-737) or photoaffinity labeled (Brunner et al., Ann. Rev. Biochem. 62:483-514, 1993 and Fedan et al., 30 Biochem. Pharmacol. 33:1167-1180, 1984) and specific cell-surface proteins can be identified.

according to conventional methods. Intravenous administration will be by bolus injection or infusion over

a typical period of one to several hours. In general, pharmaceutical formulations will include a zsig32 protein in combination with a pharmaceutically acceptable vehicle, such as saline, buffered saline, 5% dextrose in water or 5 the like. Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, etc. Methods of formulation are well known in the art and are disclosed, for example, in Remington: The Science and
10 Practice of Pharmacy, Gennaro, ed., Mack Publishing Co., Easton, PA, 19th ed., 1995. Therapeutic doses will generally determined by the clinician according to accepted standards, taking into account the nature and severity of the condition to be treated, patient traits, 15 etc. Determination of dose is within the level of ordinary skill in the art. The proteins may be administered for acute treatment, over one week or less, often over a period of one to three days or may be used in chronic treatment, over several months or years.
20 The invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1

25 Extension of EST Sequence

The novel zsig32 polypeptide-encoding polynucleotides of the present invention were initially identified by querying an EST database for secretory 30 signal sequences characterized by an upstream methionine start site, a hydrophobic region of approximately 13 amino acids and a cleavage site (SEQ ID NO: 6, wherein cleavage occurs between the alanine and glycine amino acid residues) in an effort to select for secreted proteins.
35 Polypeptides corresponding to ESTs meeting those search criteria were compared to known sequences to identify secreted proteins having homology to known ligands. A

single EST sequence was discovered and predicted to be related to a mouse ventral prostate spermine-binding protein (SPBP). See, for example, Mills et al. cited above. To identify the corresponding cDNA, a clone 5 considered likely to contain the entire coding region was used for sequencing. Using an Invitrogen S.N.A.P.™ Miniprep kit (Invitrogen, Corp., San Diego, CA) according to manufacturer's instructions a 5 ml overnight culture in LB + 50 µg/ml ampicillin was prepared. The template was 10 sequenced on an ABI PRISM™ model 377 DNA sequencer (Perkin-Elmer Cetus, Norwalk, Ct.) using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Corp.) according to manufacturer's instructions. Oligonucleotides ZC694 (SEQ ID NO: 8), 15 ZC695 (SEQ ID NO: 9) to the T7 and SP6 promoters on the clone-containing vector were used as sequencing primers. Oligonucleotides ZC13183 (SEQ ID NO: 10), ZC13187 (SEQ ID NO: 11) were used to complete the sequence from the clone. Sequencing reactions were carried out in a Hybaid OmniGene 20 Temperature Cycling System (National Labnet Co., Woodbridge, NY). SEQUENCER™ 3.0 sequence analysis software (Gene Codes Corporation, Ann Arbor, MI) was used for data analysis. The resulting 853 bp sequence is disclosed in SEQ ID NO: 1.

Example 2
Tissue Distribution

Northerns were performed using Human Multiple Tissue Blots from Clontech (Palo Alto, CA). A 40 bp DNA probe (ZC12493; SEQ ID NO: 7) to the 5' end of the oligonucleotide sequence of the mature polypeptide shown in SEQ ID NO: 1 was radioactively labeled with ³²P using T4 polynucleotide kinase and forward reaction buffer (GIBCO BRL, Gaithersburg, MD) according to the manufacturer's specifications. The probe was purified using a NUCTRAP push column (Stratagene Cloning Systems, La Jolla, CA). EXPRESSHYB (Clontech, Palo Alto, CA) solution was used for prehybridization and as a hybridizing solution for the Northern blots. Hybridization took place overnight at 42°C, and the blots were then washed in 2X SSC and 0.05% SDS at RT, followed by a wash in 1X SSC and 0.1% SDS at 71°C. One transcript size was observed at approximately 650 bp. Signal intensity was highest for prostate, stomach and trachea, with relatively less intense signals in spleen and colon.

A RNA Master Dot Blot (Clontech) that contained RNAs from various tissues that were normalized to 8 housekeeping genes was also probed with the 40 bp DNA probe (SEQ ID NO: 7). The blot was prehybridized and then hybridized overnight with 10⁶ cpm/ml of the probe at 42°C, according to the manufacturer's specifications. The blot was washed with 2X SSC and 0.05% SDS at RT, followed by a wash in 0.1X SSC and 0.1% SDS at 71°C. After a 48 hour exposure, highest expression was seen in the salivary gland, with much weaker signals in trachea and still weaker signals in prostate. Note that in prostate and trachea, a 2 kb band was also observed.

Example 3
Chromosomal Assignment and Placement of zsig32

Zsig32 was mapped to chromosome 16 using the
5 commercially available GeneBridge 4 Radiation Hybrid Panel
(Research Genetics, Inc., Huntsville, AL). The GeneBridge
4 Radiation Hybrid Panel contains PCRable DNAs from each
of 93 radiation hybrid clones, plus two control DNAs (the
HFL donor and the A23 recipient). A publicly available WWW
10 server (<http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>) allows mapping relative to the Whitehead
Institute/MIT Center for Genome Research's radiation
hybrid map of the human genome (the "WICGR" radiation
hybrid map) which was constructed with the GeneBridge 4
15 Radiation Hybrid Panel.

For the mapping of zsig32 with the GeneBridge 4
RH Panel, 20 μ l reactions were set up in a 96-well
microtiter plate (Stratagene) and used in a RoboCycler
Gradient 96 thermal cycler (Stratagene). Each of the 95
20 PCR reactions consisted of 2 μ l 10X KlenTaq PCR reaction
buffer (Clontech), 1.6 μ l dNTPs mix (2.5 mM each, PERKIN-
ELMER, Foster City, CA), 1 μ l sense primer, ZC 13,703 (SEQ
ID NO:21), 1 μ l antisense primer, ZC 13,704 (SEQ ID
NO:22), 2 μ l RediLoad (Research Genetics, Inc.), 0.4 μ l
25 50X Advantage KlenTaq Polymerase Mix (Clontech
Laboratories, Inc.), 25 ng of DNA from an individual
hybrid clone or control and ddH₂O for a total volume of 20
 μ l. The reactions were overlaid with an equal amount of
mineral oil and sealed. The PCR cycler conditions were as
30 follows: an initial 1 cycle 5 minute denaturation at 95°C,
35 cycles of a 1 minute denaturation at 95°C, 1 minute
annealing at 60°C and 1.5 minute extension at 72°C,

.....

The results showed that Zsig32 maps 7.47 cR_3000
from the framework marker WT- 7742 on the WICGR chromosome.

16 radiation hybrid map. Proximal and distal framework
markers were WI-7742 (D16S2960) and WI-3061 (D16S2965),
respectively. The use of surrounding markers positions
Zsig32 in the 16p13.3 region on the integrated LDB
5 chromosome 16 map (The Genetic Location Database,
University of Southampton, WWW server:
http://cedar.genetics.soton.ac.uk/public_html/).

10

Example 4Creation of mammalian expression vectors
zsig32NF/pZP9, zsig32CF/pZP9 and zsig32/pZP9

15 Three expression vectors were prepared for the
zsig32 polypeptide, zSIG32CF/pZP9 and zSIG32NF/pZP9,
wherein the constructs are designed to express a zsig25
polypeptide with a C- or N-terminal FLAG tag (SEQ ID NO:
16) and zSIG32/pZP9 expressing untagged zsig32
polypeptides.

20

ZSIG32/pZP9

25 A approximately 800 bp restriction digest
fragment of ZSIG-32 DNA was derived from the clone
described in Example 1 above. Ten microliters of the
clone was digested with 1.5 μ l each of the restriction
enzymes Eco RI and Not I. The resultant ligation
fragment was then run on a 0.8% LMP agarose gel (Seaplaque
GTG) with 0.5x TBE buffer. A band of the predicted size
was excised and the DNA was purified from the gel with a
QIAQUICK[®] column (Qiagen) according the manufacturer's
instructions.

30

35 The excised, restriction digested zsig32 DNA was
subcloned into plasmid pZP9 which had been cut with Eco RI
and Not I. Plasmid CF/pZP9 (deposited at the American
Type Culture Collection, 12301 Parklawn Drive, Rockville,
MD) is a mammalian expression vector containing an
expression cassette having the mouse metallothionein-1
promoter, multiple restriction sites for insertion of
coding sequences, a stop codon and a human growth hormone

terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene and the SV40 terminator.

5

zSIG25CF/pZP9

A 553 bp PCR generated ZSIG-32 DNA fragment was created using ZC13465 (SEQ ID NO:23) and ZC13447 (SEQ ID NO:26) as PCR primers and the template described in 10 Example 1 above. The PCR reaction was incubated at 94°C for 5 minutes, and then run for 10 cycles of 30 seconds at 94°C and 2 minutes at 72°C, followed by 15 cycles at 94°C for 30 seconds and 65°C for 2 minutes. The resultant PCR product was then run on a 0.9% GTG/TBE agarose gel with 1x 15 TBE buffer. A band of the predicted size was excised and the DNA was purified from the gel with a QIAQUICK[®] column (Qiagen) according the manufacturer's instructions. The DNA was digested with the restriction enzymes BAM HI (Boehringer Mannheim) and Eco RI (Gibco BRL), followed by 20 phenol/chloroform/isoamyl alcohol extraction and ETOH/glycogen precipitated.

The excised, restriction digested zsig32 DNA was subcloned into plasmid CF/pZP9 which had been cut with Eco RI and Bam HI. The zSIG32/CFpZP9 expression vector uses 25 the native zSIG32 signal peptide, and the FLAG epitope (SEQ ID NO:16) is attached at the C-terminus as a purification aid. Plasmid CF/pZP9 (deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD) is a mammalian expression vector containing 30 an expression cassette having the mouse metallothionein-1 promoter, multiple restriction sites for insertion of

replication, a mammalian selectable marker expression unit

having an SV40 promoter, enhancer and origin of replication, a DHFR gene and the SV40 terminator.

zSIG25NF/pZP9

5 A 490 bp PCR generated zSIG32/NF DNA fragment was created in accordance with the procedure set forth above using Z13448 (SEQ ID NO:25) and ZC13449 (SEQ ID NO:26) as PCR primers. The purified PCR fragment was digested with the restriction enzymes Bam HI (Boehringer 10 Mannheim) and Xho I (Gibco BRL), followed by phenol/chloroform/isoamyl alcohol extraction and ETOH/glycogen precipitation.

The excised and restriction digested zSIG32 DNA was subcloned into plasmid NF/pZP9 which had been cut with 15 Bam HI and Xba I. The zSIG32/NFpZP9 expression vector incorporates the TPA leader and attaches the FLAG tag (SEQ ID NO:16) to the N-terminal of the zsig25 polypeptide-encoding polynucleotide sequence. Plasmid NF/pZP9 (deposited at the American Type Culture Collection, 12301 20 Parklawn Drive, Rockville, MD) is a mammalian expression vector containing an expression cassette having the mouse metallothionein-1 promoter, a TPA leader peptide followed by the sequence encoding the FLAG tag (SEQ ID NO:16), multiple restriction sites for insertion of coding 25 sequences, and a human growth hormone terminator. The plasmid also contains an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene and the SV40 terminator.

30 For the untagged zsig32 construct 100 ng of the zsig32 insert and 90 ng of the Eco RI/Not I digested pZP9 vector were ligated as described for the tagged constructs. For the N- and C-tagged constructs, 10 ng of the restriction digested inserts and 20 ng of the 35 corresponding vectors were ligated at room temperature for 4 hours. One microliter of each ligation reaction was

independently electroporated into DH10B competent cells (GIBCO BRL, Gaithersburg, MD) according to manufacturer's direction and plated onto LB plates containing 50 mg/ml ampicillin, and incubated overnight. Colonies were 5 screened by PCR as described above. For zsig32/pZP9 screens the primers were ZC6583 (SEQ ID NO:27) and ZC5020 (SEQ ID NO:28), for zSIG32CF/pZP9 screens the primers were, ZC13465 (SEQ ID NO:23) and ZC13447 (SEQ ID NO:24) and for zSIG32NF/pZP9 screens the primers were ZC13448 10 (SEQ ID NO:25) and ZC13449 (SEQ ID NO:26). The insert sequence of positive clones, 1000 bp for zsig32 untagged, 490 bp fragment for zSIG32NF and a 553 bp fragment for zSIG32/CF were verified by sequence analysis. A large scale plasmid preparation was done using a QIAGEN[®] Maxi 15 prep kit (Qiagen) according to manufacturer's instructions.

Example 5
Mammalian Expression of zsig32

20 BHK 570 cells (ATCC NO: CRL-10314) were plated in 10 cm tissue culture dishes and allowed to grow to approximately 50 to 70% confluency overnight at 37°C, 5% CO₂, in DMEM/FBS media (DMEM, Gibco/BRL High Glucose, 25 Gibco BRL, Gaithersburg, MD), 5% fetal bovine serum (Hyclone, Logan, UT), 1 µM L-glutamine (JRH Biosciences, Lenexa, KS), 1 µM sodium pyruvate (Gibco BRL)). The cells were then transfected with the plasmid zsig32NF/pZP9 (N-terminal FLAG tag), zsig32CF/pZP9 (C-terminal FLAG tag), 30 or zsig32/pZP9 (untagged), using LipofectamineTM (Gibco BRL), in serum free (SF) media formulation (DMEM, 10 mg/ml transferrin, 5 mg/ml insulin, 2 mg/ml fetuin, 1% L-

with SF media. In separate tubes, 35 µl of LipofectamineTM (Gibco BRL) was mixed with 605 µl of SF medium. The

Lipofectamine™ mix was added to the DNA mix and allowed to incubate approximately 30 minutes at room temperature. Five milliliters of SF media was added to the DNA:Lipofectamine™ mixture. Three plates of cells were 5 rinsed once with 5 ml of SF media, aspirated, and the DNA:Lipofectamine™ mixture was added. The cells were incubated at 37°C for five hours, then 6.4 ml of DMEM/10% FBS, 1% PSN media was added to each plate. The plates were 10 incubated at 37°C overnight and the DNA:Lipofectamine™ mixture was replaced with fresh FBS/DMEM media the next day. On day 2 post-transfection, the cells were split into the selection media (DMEM/FBS media from above with the addition of 1 µM methotrexate (Sigma Chemical Co., St. Louis, Mo.)) in 150 mm plates at 15 1:10, 1:20 and 1:50. The cells were refed at day 5 post-transfection with fresh selection media. Approximately 12 days post-transfection, two 150 mm culture dishes of methotrexate resistant colonies from each transfection were trypsinized and the cells were pooled and plated into 20 a T-162 flask and transferred to large scale culture.

Example 6
Large Scale Mammalian Expression of zsig32

25 One T-162 flask, containing confluent cells expressing zsig32/NF and one flask containing zsig32/CF expressing cells, obtained from the expression procedure described above, were expanded into five T-162 flasks. One of the five resulting flasks was used to freeze down 30 four cryovials, and the other four flasks were used to generate a Nunc cell factory.

The cells from the four T-165 flasks of zsig32/NF and zsig32/CF were combined and used to seed two Nunc cell factories (10 layers, commercially available 35 from VWR). Briefly, the cells from the T-162 flasks described above were detached using trypsin, pooled, and added to 1.5 liters ESTEP1 media (668.7g/50L DMEM (Gibco),

5.5 g/50L pyruvic acid, sodium salt 96% (Mallinckrodt), 185.0 g/50L NaHCO₃ (Mallinckrodt), 5.0 mg/ml and 25 ml/50L insulin (JRH Biosciences), 10.0 mg/ml and 25 ml/50L transferrin (JRH Biosciences), 2.5L/50L fetal bovine serum (characterized) (Hyclone), 1 μ M MTX, with pH adjusted to 7.05 +/- 0.05) prewarmed to 37°C. The media containing the cells was then poured into the Nunc cell factories via a funnel. The cell factories were placed in a 37°C/5.0% CO₂ incubator.

10 At 80-100% confluence, a visual contamination test (phenol red color change) was performed on the Nunc cell factories. Since no contamination was observed, supernatant from the confluent factories was poured into a small harvest container, sampled and discarded. The 15 adherent cells were then washed once with 400 ml PBS. To detach the cells from the factories, 100 mls of trypsin was added to each and removed and the cells were then incubated for 5 to 10 minutes in the residual trypsin. The cells were collected following two, 200 ml washes of 20 ESTEP1 media. To each of ten ESTEP1 media-containing bottles (1.5 liters each, at 37°C) was added 40 mls of collected cells. One 1.5 liter bottle was then used to fill one Nunc factory. Each cell factory was placed in a 37°C/5.0% CO₂ incubator.

25 At 80-90% confluence, a visual contamination test (phenol red color change) was performed on the Nunc cell factories. Since no contamination was observed, supernatant from the confluent factories was poured into a small harvest container, sampled and discarded. Cells 30 were then washed once with 400 ml PBS. 1.5 liters of ESTEP2 media (668.7g/50L DMEM (Gibco), 5.5 g/50L pyruvic

factory. The cell factories were incubated at 37°C/5.0% CO₂.

At approximately 48 hours a visual contamination test (phenol red color change) was performed on the Nunc cell factories. Supernatant from each factory was poured into small harvest containers. A total of 13.5 liters was 5 collected from all 10 factories. Fresh serum-free media (1.5 liters) was poured into each Nunc cell factory, and the factories were incubated at 37°C/5.0% CO₂. One ml of supernatant harvest was transferred to a microscope slide, and subjected to microscopic analysis for contamination.

10 The contents of the small harvest containers for each factory were pooled and immediately filtered. A second harvest was then performed, substantially as described above at 44 hours (13.5 L were obtained) and the cell factories were discarded thereafter. An aseptically

15 assembled filter train apparatus was used for aseptic filtration of the harvest supernatant (conditioned media). Assembly was as follows: tubing was wire-tied to an Opti-Cap filter (Millipore Corp., Bedford, MA) and a Gelman Supercap 50 filter (Gelman Sciences, Ann Arbor, MI). The

20 Supercap 50 filter was also attached to a sterile capped container located in a hood; tubing located upstream of the Millipore Opti-cap filter was inserted into a peristaltic pump; and the free end of the tubing was placed in the large harvest container. The peristaltic

25 pump was run between 200 and 300 rpm, until all of the conditioned media passed through the 0.22 µm final filter into a sterile collection container. The filtrate was placed in a 4 °C cold room pending purification. The media was concentrated 10X with a Millipore 5 kDA cut off

30 concentrator (Millipore Corp., Bedford, MA) according to manufacturer's direction and subjected to Western Blot analysis using an anti-FLAG tag antibody (Kodak).

Example 7
Purification of Mammalian Expressed FLAG-tagged zsig32
Polypeptides

Unless otherwise noted, all operations were carried out at 4°C. The following procedure was used to purify zsig32 constructs having an N-terminal or C-terminal flag tag. Protein was purified from the culture medium of a mixture of baby hamster kidney cell (BHK) clones that produced the N- or C-terminal tagged protein (zsig32NF and zsig32CF). A total of 25 liters of conditioned media from BHK cells was sequentially sterile filtered through a 4 inch, 0.2 mM Millipore (Bedford, MA) OptiCap capsule filter and a 0.2 mM Gelman (Ann Arbor, MI) Supercap 50. The material was then concentrated to about 1.3 liters using an Amicon (Beverly, MA) DC 10L concentrator fitted with an A/G Tech (Needham, MA) hollow fiber cartridge with a 15 sq. ft. 3000 kDa cutoff membrane. The concentrated material was again sterile-filtered with the Gelman filter as described above. A 25.0 ml sample of anti-Flag Sepharose (Eastman Kodak, Rochester, NY) was added to the sample for batch adsorption and the mixture was gently agitated on a Wheaton (Millville, NJ) roller culture apparatus for 18.0 h at 4°C.

The mixture was then poured into a 5.0 x 20.0 cm Econo-Column (Bio-Rad, Laboratories, Hercules, CA) and the gel was washed with 30 column volumes of phosphate buffered saline (PBS). The unretained flow-through fraction was discarded. Once the absorbance of the effluent at 280 nM was less than 0.05, flow through the column was reduced to zero and the anti-Flag Sepharose gel was washed with 20 column volumes of PBS containing 0.1

flow was resumed and the eluted protein was collected. This fraction is referred to as the peptide elution. The

of 0.1M glycine, pH 2.5, and the glycine wash was collected separately. The pH of the glycine-eluted fraction was adjusted to 7.0 by the addition of a small volume of 10X PBS and stored at 4°C for future analysis if 5 needed.

The peptide elution was concentrated to 5.0 ml using a 5,000 molecular weight cutoff membrane concentrator (Millipore, Bedford, MA) according to the manufacturer's instructions. The concentrated peptide 10 elution was then separated from free peptide by chromatography on a 1.5 x 50 cm Sephadex G-50 (Pharmacia, Piscataway, NJ) column equilibrated in PBS at a flow rate of 1.0 ml/min using a BioCad Sprint HPLC system (PerSeptive BioSystems, Framingham, MA). Two-ml fractions 15 were collected and the absorbance at 280 nM was monitored. The first peak of material absorbing at 280 nM and eluting near the void volume of the column was collected.

By SDS-PAGE and Western analysis with anti-Flag M2 antibodies (Kodak) the purified zsig32 NF/CF 20 preparation was composed of one major Coomassie Blue-stained band of apparent molecular weight 25,000. Two minor bands of apparent molecular weights 22,000 and 27,000, were also observed on the Coomassie Blue-stained gel. All three bands showed cross-reactivity with the 25 anti-Flag M2 antibody on Western blots. Migration of the proteins on SDS-PAGE gels and Western blots was not changed by reducing agents.

The protein concentration of the purified proteins (0.67 mg/ml) was determined by BCA analysis 30 (Pierce, Rockford, IL) and the material was aliquoted, and stored at -80°C.

Example 8

35 Creation of baculovirus expression vectors
pFSG32, pFSGE32, pSSGE32 and pLSGE32

Four expression vectors were prepared to express zsig32 polypeptides in insect cells: pFSG32, designed to

express an untagged zsig32 polypeptide; and pFSGE32, pSSGE32 and pLSGE32, designed to express a zsig32 polypeptide with a C-terminal Glu-Glu tag (SEQ ID NO:12).

5

pFSG32

A 559 bp PCR generated zsig32 DNA fragment was created using ZC13404 (SEQ ID NO:13) and ZC13409 (SEQ ID NO:14) as PCR primers and zsig32/pZP9, described above, as a template. The PCR reaction was incubated at 94°C for 2 minutes, followed by 30 cycles of 45 seconds at 94°C, 1 minute at 60°C and 72°C for 1 minute with a 1 second/cycle segment extension. The resultant PCR product was then run on a 3% gel (2% NuSieve/1% BRL agarose). The 559 bp fragment was captured by diluting 15 fold with 0.1 mM EDTA pH 8.0 and then ligated into the vector pCR2.1 (TA Cloning Kit, Invitrogen Inc., San Diego, CA) according to manufacturer's instructions. The resultant clones were screened for the proper insert orientation and sequenced to confirm identity. The resulting clone, designated 10 pSG32a, was digested with Bam HI and Asp718 and the digest run on a 1% SeaPlaque/1% NuSieve agarose gel. The band was excised, diluted to 0.5% agarose with 2 mM MgCl₂, melted at 65°C and ligated into a Bam HI/Asp718 digested baculovirus expression vector, pFastBac1 (Bac-to-Bac™ System, GIBCO-BRL, Gaithersburg, MD). Forty four 15 nanograms of the restriction digested zsig32 insert and 126 ng of the corresponding vector were ligated overnight. The ligation mix was diluted 3 fold in TE (10 mM Tris-HCl, pH 7.5 and 1 mM EDTA) and 4 fmol of the diluted ligation 20 mix was transformed into DH5α Library Efficiency competent cells (Life Technologies) according to manufacturer's

instructions. The transformants were plated onto LB medium.

IM NaCl, 1.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, and 20 mM glucose) and plated onto LB plates containing 100 µg/ml

ampicillin. The plates were incubated overnight at 37°C. Plasmid DNA was prepared using the QiaVac Miniprep8 system (Qiagen) according the manufacturer's directions. The clones were screened by restriction digest with Hind III/BspE1.

pFSGE32

A zsig32 fragment having a C-terminal Glu-Glu tag (SEQ ID NO:12) was generated by PCR as described above using oligonucleotide primers ZC13404 (SEQ ID NO:13) and 10 ZC13407 (SEQ ID NO:15). A fragment of the expected size, 580 bp, was detected by gel electrophoresis. The DNA fragment was digested with the restriction enzymes Bam HI and Asp718 and the resulting 565 bp zsig32 restriction fragment was ligated into a Bam HI/Asp718 digested 15 pFastBac1 vector and transformed into DH10 α cells as described above.

pSSGE32 and pLSGE32

A 580 bp zsig32 fragment having a C-terminal Glu-Glu tag (SEQ ID NO:12) was generated by PCR as 20 described above. The fragment was visualized by gel electrophoresis, restriction digested and ligated into the expression vectors, pFBPL2 and pFBPS2, as described above. The vectors, pFBPL2 and pFBPS2, were derived from a modified pFastBac1™ vector. The polyhedrin promoter was 25 removed and substituted with a short (FBPS) or long (FBPL) version of the baculovirus basic protein promoter as is known in the art (Hill-Perkins and Possee, *ibid.*; Bonning et al., *ibid.*; and, Chazenbalk and Rapoport, *ibid.*).

One microliter of each of the above constructs 30 was used to independently transform 20 μ l DH10Bac Max Efficiency competent cells (GIBCO-BRL, Gaithersburg, MD) according to manufacturer's instruction, by heat shock at 42°C for 45 seconds. The transformants were then diluted in an appropriate volume of SOC media and plated on to 35 Luria Agar plates containing 50 μ g/ml kanamycin, 7 μ g/ml gentamicin, 10 μ g/ml tetracycline, IPTG and Bluo Gal. The

cells were incubated for 48 hours at 37°C. A color selection was used to identify those cells having virus that had incorporated into the plasmid (referred to as a "bacmid"). Those colonies, which were white in color, 5 were picked for analysis. Bacmid DNA was isolated from positive colonies and screened for the correct insert using PCR. Oligonucleotide primers ZC976 (SEQ ID NO:17) and ZC447 (SEQ ID NO:18) were used and those having the correct insert were used to transfect *Spodoptera frugiperda* (Sf9) cells. 10

Sf9 cells were seeded at 5×10^6 cells per 35 mm plate and allowed to attach for 1 hour at 27°C. Five microliters of bacmid DNA was diluted with 100 μ l Sf-900 II SFM. Six to 10 μ l of CellFECTIN Reagent (Life 15 Technologies) was diluted with 100 μ l Sf-900 II SFM. The bacmid DNA and lipid solutions were gently mixed and incubated 30-45 minutes at room temperature. The media from one plate of cells were aspirated, and the lipid-DNA mixture to which 0.8 ml of Sf-900 II SFM was added. The media 20 cells were incubated at 27°C for 4-5 hours, then 2 ml of Sf-900 II media containing penicillin/streptomycin was added to each plate. The plates were incubated at 27°C, 90% humidity, for 72 hours after which the virus was harvested.

25 Primary Amplification

Sf9 cells were grown in 50 ml Sf-900 II SFM in a 50 ml shake flask to an approximate density of 0.04-0.50 $\times 10^6$ cells/ml. They were then transfected with 50-1000 μ l of the virus stock from above and incubated at 27°C for 3-30 5 days after which time the virus was harvested, titer 0.53×10^8 pfu/ml. To scale up, 1.5×10^6 SF9 cells/ml

Example 9
Purification of Baculovirus Expressed Glu-Glu-tagged
zsig32 polypeptides

5 Unless otherwise noted, all operations were
carried out at 4°C. A mixture of protease inhibitors were
added to a 2 liter sample of conditioned media from C-
terminal Glu-Glu (EE) tagged zsig32 baculovirus-infected
Sf9 cells to final concentrations of 2.5 mM
10 ethylenediaminetetraacetic acid (EDTA, Sigma Chemical Co.
St. Louis, MO), 0.001 mM leupeptin (Boehringer-Mannheim,
Indianapolis, IN), 0.001 mM pepstatin (Boehringer-
Mannheim) and 0.4 mM Pefabloc (Boehringer-Mannheim). The
sample was centrifuged at 10,000 rpm for 30 min at 4°C in
15 a Beckman JLA-10.5 rotor (Beckman Instruments) in a
Beckman Avanti J25I centrifuge (Beckman Instruments) to
remove cell debris. To the supernatant fraction was added
a 50.0 ml sample of anti-EE Sepharose, prepared as
described below, and the mixture was gently agitated on a
20 Wheaton (Millville, NJ) roller culture apparatus for 18.0
h at 4°C.

25 The mixture was poured into a 5.0 x 20.0 cm
Econo-Column (Bio-Rad Laboratories) and the gel was washed
with 30 column volumes of phosphate buffered saline (PBS).
The unretained flow-through fraction was discarded. Once
the absorbance of the effluent at 280 nM was less than
0.05, flow through the column was reduced to zero and
the anti-EE Sepharose gel was washed with 2.0 column
30 volumes of PBS containing 0.2 mg/ml of EE peptide
(AnaSpec, San Jose, CA). The peptide used has the
sequence Glu-Tyr-Met-Pro-Val-Asp (SEQ ID NO: 19). After
1.0 hour at 4°C, flow was resumed and the eluted protein
was collected. This fraction was referred to as the
peptide elution. The anti-EE Sepharose gel was washed
35 with 2.0 column volumes of 0.1 M glycine, pH 2.5, and the
glycine wash was collected separately. The pH of the

glycine-eluted fraction was adjusted to 7.0 by the addition of a small volume of 10X PBS and stored at 4°C.

The peptide elution was concentrated to 5.0 ml using a 5,000 molecular weight cutoff membrane 5 concentrator (Millipore) according to the manufacturer's instructions. The concentrated peptide elution was separated from free peptide by chromatography on a 1.5 x 50 cm Sephadex G-50 (Pharmacia) column equilibrated in PBS at a flow rate of 1.0 ml/min using a BioCad Sprint HPLC 10 (PerSeptive BioSystems). Two ml fractions were collected and the absorbance at 280 nM was monitored. The first peak of material absorbing at 280 nM and eluting near the void volume of the column was collected. This material represented purified zsig32CEE and was composed of two 15 major bands of apparent molecular weights 19,000 and 24,000 on Coomassie Blue-stained SDS-PAGE gels. These bands were present in about equimolar amounts. Both bands showed cross-reactivity with anti-EE antibodies by Western blotting of the purified material. The protein 20 concentration (0.53 mg/ml) of the purified proteins was determined by BCA analysis (Pierce) and the material was aliquoted, and stored at -80°C.

Preparation of anti-EE Sepharose

25 A 100 ml bed volume of protein G-Sepharose (Pharmacia) was washed 3 times with 100 ml of PBS containing 0.02% sodium azide using a 500 ml Nalgene 0.45 micron filter unit. The gel was washed with 6.0 volumes of 200 mM triethanolamine, pH 8.2 (TEA, Sigma), and an 30 equal volume of EE antibody solution containing 900 mg of antibody was added. After an overnight incubation at 4°C,

35 a 100 ml volume of 1M, 1,10-phenanthroline, a suitable container, and dimethylpimelimidate-2 HCl (Pierce), dissolved in TEA, was added to a final

concentration of 36 mg/ml of gel. The gel was rocked at room temperature for 45 min and the liquid was removed using the filter unit as described above. Nonspecific sites on the gel were then blocked by incubating for 10 5 minutes at room temperature with 5 volumes of 20 mM ethanalamine in 200 mM TEA. The gel was then washed with 5 volumes of PBS containing 0.02% sodium azide and stored in this solution at 4°C.

10

Example 9Construction of zsig32 Amino Terminal FLAG-Tagged
Pichia Expression Vector

15 Expression of zsig32 in *Pichia methanolica* utilizes the expression system described in co-assigned WIPO publication WO 97/17450. An expression plasmid containing all or part of a polynucleotide encoding zsig32 was constructed via homologous recombination. The expression vector was built from pCZR190, which contains 20 the AUG1 promoter, followed by the alpha factor prepro (α Fpp) leader sequence, followed by an amino-terminal FLAG tag (NF), a blunt-ended Sma I restriction site for insertion of the gene sequence of interest, a translational stop codon, followed by the AUG1 terminator, 25 the ADE2 selectable marker, and finally the AUG1 3' untranslated region. Also included in this vector are the URA3 and CEN-ARS sequences required for selection and replication in *S. cerevisiae*, and the AmpR and colE1 ori sequences required for selection and replication in *E. coli*. The zsig32 sequence inserted into this vector 30 begins at residue 22 (Ala) of the zsig32 amino acid sequence (SEQ ID NO:2).

35 Two construct specific linkers were prepared and along with zsig32, were homologously recombined into the yeast expression vector pCZR190. The N-terminal linker comprises 70 base pairs of the α Fpp coding sequence joined

to a nucleotide sequence encoding a FLAG tag (SEQ ID NO:16) followed by 70 base pairs of nucleotide sequence encoding a portion of the amino-terminus from the mature zsig32 sequence. The C-terminal linker comprises about 70 base pairs of carboxy terminus coding sequence of the zsig32 joined with 70 base pairs of AUG1 terminator sequence.

The N-terminal linker was synthesized by a PCR reaction. Briefly, to a final reaction volume of 100 μ l was added 1 pm each of each linker ZC13735 (SEQ ID NO:29), ZC14291 (SEQ ID NO:30), and 100 pmol of each primer ZC13497 (SEQ ID NO:31) and ZC14279 (SEQ ID NO:32), 10 μ l of 10X PCR buffer (Boehringer Mannheim), 1 μ l Two polymerase (Boehringer Mannheim), 10 μ l of 0.25 mM nucleotide triphosphate mix (Perkin Elmer) and dH₂O. The PCR reaction was incubated at 94°C for 1.5 minutes, followed by 10 cycles of 30 seconds at 94°C, 1 minute at 50°C and 1 minute at 72°C, concluded with a 10 minute extension at 72°. The resulting 138 bp double stranded, NF-tagged linker is disclosed in SEQ ID NO:33.

The C-terminal untagged zsig32 linker was made via a PCR reaction as described using oligonucleotides linkers ZC14346 (SEQ ID NO:34) and ZC14218 (SEQ ID NO:35) and primers ZC14278 (SEQ ID NO:36) and ZC13734 (SEQ ID NO:37). The resulting 153 bp double stranded, C-terminal untagged linker is disclosed in SEQ ID NO:38.

The NF-zsig32 plasmid was made by homologously recombining 100 ng of Sma I digested pCZR202 acceptor vector, the 1 μ g of Eco RI-Xho I zsig32 cDNA donor fragment, 1 μ g of N-terminal FLAG-tagged zsig32 linker (SEQ ID NO:33) and 1 μ g of C-terminal linker (SEQ ID

1) a 0.1 cm electroporation cuvette. The yeast/DNA mixture was electropulsed at 0.75 kV (5 kV/cm), ∞ ohms, 25 μ F. To

the cuvette was added 600 μ l of 1.2 M sorbitol and 300 μ l aliquots of the yeast/sorbitol mixture was plated onto two URA D plates and incubated at 30°C.

After about 48 hours the Ura⁺ yeast transformants 5 from a single plate were resuspended in 2.5 ml H₂O and spun briefly to pellet the yeast cells. The cell pellet was resuspended in 1 ml of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA). Five hundred microliters of the lysis mixture was added to an 10 Eppendorf tube containing 300 μ l acid washed glass beads and 200 μ l phenol-chloroform, vortexed for 1 minute intervals two or three times, followed by a 5 minute spin in a Eppendorf centrifuge at maximum speed. Three hundred microliters of the aqueous phase was transferred to a 15 fresh tube and the DNA precipitated with 600 μ l ethanol (EtOH), followed by a 10 minutes at 4°C. The DNA pellet was resuspended in 100 μ l H₂O.

Five microliters of the resuspended DNA prep was used to transform 40 μ l of electrocompetent *E. coli* cells 20 (DH10B, Gibco BRL). The cells were electropulsed at 2.0 kV and 400 ohms. Following electroporation, 1 ml SOC (2% BactoTM Tryptone (Difco, Detroit, MI), 0.5% yeast extract (Difco), 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 25 20 mM glucose) was added and the cells were allowed to recover for 1 hour at 37°C prior to plating 250 μ l aliquots on four LB AMP plates (LB broth (Lennox), 1.8% BactoTM Agar (Difco), 100 mg/L Ampicillin).

Individual clones harboring the correct expression construct were identified by PCR screening. 30 The primers used to amplify the N-tagged zsig32 clone were ZC13479 (SEQ ID NO:31) and ZC13734 (SEQ ID NO:37). The insert sequence of positive clones, identified by a 625 bp fragment, were verified by sequence analysis. One such clone was designated pGMN12-1. Larger scale plasmid DNA 35 was isolated using Qiagen maxi kits (Qiagen) and the DNA was digested with Not I to liberate the *Pichia*-zsig32

expression cassette from the vector backbone. The Not I DNA fragment was then transformed into the *Pichia methanolica* expression host, PMAD16. This was done by mixing 100 μ l of prepared competent PMAD16 cells with 10 5 μ g of Not I digested NF-tagged zsig32 fragment and transferred to a 0.2 cm electroporation cuvette. The yeast/DNA mixture was electropulsed at 0.75 kV, 25 μ F, infinite ohms. To the cuvette was added 1 ml of 1X Yeast Nitrogen Base and 500 μ l aliquots were plated onto two ADE 10 DS (0.056% -Ade -Trp -Thr powder, 0.67% yeast nitrogen base without amino acids, 2% D-glucose, 0.5% 200X tryptophan, threonine solution, and 18.22% D-sorbitol) plates for selection and incubated at 30°C. The resulting NF-tagged-zsig32 plasmid was designated 15 PMAD16::pGMN12-1. Transformants were then picked and screened via Western blot for high-level NF-tagged zsig32 expression and subjected to large scale fermentation.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been 20 described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: ZymoGenetics, Inc.
1201 Eastlake Ave. E.
Seattle
WA
USA
98102

(ii) TITLE OF THE INVENTION: SECRETED SALIVARY ZSIG32
POLYPEPTIDES

(iii) NUMBER OF SEQUENCES: 38

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: ZymoGenetics
(B) STREET: 1201 Eastlake Ave. E.
(C) CITY: Seattle
(D) STATE: WA
(E) COUNTRY: USA
(F) ZIP: 98102

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 60/041,263
(B) FILING DATE: March 19, 1997

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Lingenfelter, Susan E
(B) REGISTRATION NUMBER: 41.156
(C) REFERENCE/DOCKET NUMBER: 97-17PC

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 206-442-6675
- (B) TELEFAX: 206-442-6678
- (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 853 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 168...701
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

| | | | | | | |
|-------------|------------|------------|------------|------------|-------------|-----|
| GAATTCTGGCT | CGAGAGGAAG | AGCCCCACGG | CCAGCTCCTT | CCTGTTCCCC | TGGCGGCC | 60 |
| TCGCTTCTTC | CTTCTGGATG | GGGGCCAGG | GGGCCAGGA | GAGTATAAAG | GCGATGTGGA | 120 |
| GGGTGCCCGG | CACAACCAGA | CGCCCAGTCA | CAGGCGAGAG | CCCTGGG | ATG CAC CGG | 176 |
| | | | | | Met His Arg | |
| | | | | | 1 | |
| CCA | GAG | GCC | ATG | CTG | CTG | 224 |
| Pro | Glu | Ala | Met | Leu | Leu | |
| 5 | 10 | 15 | | | | |
| ACC | TGG | GCA | GGG | AAG | ATG | 272 |
| Thr | Trp | Ala | Gly | Lys | TAT | |
| 20 | 25 | 30 | 35 | | | |

| | | | |
|--|-----|-----|-----|
| GGT CTT CTC CTG GTG AAA AGT GTC CAG GTG AAA CTT GGA GAC TCC TGG | 368 | | |
| Gly Leu Leu Leu Val Lys Ser Val Gln Val Lys Leu Gly Asp Ser Trp | | | |
| 55 | 60 | 65 | |
| GAC GTG AAA CTG GGA GCC TTA GGT GGG AAT ACC CAG GAA GTC ACC CTG | 416 | | |
| Asp Val Lys Leu Gly Ala Leu Gly Gly Asn Thr Gln Glu Val Thr Leu | | | |
| 70 | 75 | 80 | |
| CAG CCA GGC GAA TAC ATC ACA AAA GTC TTT GTC GCC TTC CAA GCT TTC | 464 | | |
| Gln Pro Gly Glu Tyr Ile Thr Lys Val Phe Val Ala Phe Gln Ala Phe | | | |
| 85 | 90 | 95 | |
| CTC CGG GGT ATG GTC ATG TAC ACC AGC AAG GAC CGC TAT TTC TAT TTT | 512 | | |
| Leu Arg Gly Met Val Met Tyr Thr Ser Lys Asp Arg Tyr Phe Tyr Phe | | | |
| 100 | 105 | 110 | 115 |
| GGG AAG CTT GAT GGC CAG ATC TCC TCT GCC TAC CCC AGC CAA GAG GGG | 560 | | |
| Gly Lys Leu Asp Gly Gln Ile Ser Ser Ala Tyr Pro Ser Gln Glu Gly | | | |
| 120 | 125 | 130 | |
| CAG GTG CTG GTG GGC ATC TAT GGC CAG TAT CAA CTC CTT GGC ATC AAG | 608 | | |
| Gln Val Leu Val Gly Ile Tyr Gly Gln Tyr Gln Leu Leu Gly Ile Lys | | | |
| 135 | 140 | 145 | |
| AGC ATT GGC TTT GAA TGG AAT TAT CCA CTA GAG GAG CCG ACC ACT GAG | 656 | | |
| Ser Ile Gly Phe Glu Trp Asn Tyr Pro Leu Glu Glu Pro Thr Thr Glu | | | |
| 150 | 155 | 160 | |
| CCA CCA GTT AAT CTC ACA TAC TCA GCA AAC TCA CCC GTG GGT CGC TAGGG | 706 | | |
| Pro Pro Val Asn Leu Thr Tyr Ser Ala Asn Ser Pro Val Gly Arg | | | |
| 165 | 170 | 175 | |
| TGGGGTATGG GGCCATCCGA GCTGAGGCCA TCTGTGTGGT GGTGGCTGAT GGTACTGGAG | 766 | | |
| TAAC TGAGTC GGGACGCTGA ATCTGAATCC ACCAATAAAT AAAGCTTCTG CAGAATCAGT | 826 | | |
| GAAAAAAA AAAAAAAGGG CGGCCGC | 853 | | |

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 178 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(x) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met His Arg Pro Glu Ala Met Leu Leu Leu Thr Leu Ala Leu Leu
 1 5 10 15
 Gly Gly Pro Thr Trp Ala Gly Lys Met Tyr Gly Pro Gly Gly Lys
 20 25 30
 Tyr Phe Ser Thr Thr Glu Asp Tyr Asp His Glu Ile Thr Gly Leu Arg
 35 40 45
 Val Ser Val Gly Leu Leu Leu Val Lys Ser Val Gln Val Lys Leu Gly
 50 55 60
 Asp Ser Trp Asp Val Lys Leu Gly Ala Leu Gly Gly Asn Thr Gln Glu
 65 70 75 80
 Val Thr Leu Gln Pro Gly Glu Tyr Ile Thr Lys Val Phe Val Ala Phe
 85 90 95
 Gln Ala Phe Leu Arg Gly Met Val Met Tyr Thr Ser Lys Asp Arg Tyr
 100 105 110
 Phe Tyr Phe Gly Lys Leu Asp Gly Gln Ile Ser Ser Ala Tyr Pro Ser
 115 120 125
 Gln Glu Gly Gln Val Leu Val Gly Ile Tyr Gly Gln Tyr Gln Leu Leu
 130 135 140
 Gly Ile Lys Ser Ile Gly Phe Glu Trp Asn Tyr Pro Leu Glu Glu Pro
 145 150 155 160
 Thr Thr Glu Pro Pro Val Asn Leu Thr Tyr Ser Ala Asn Ser Pro Val
 165 170 175
 Gly Arg

(2) INFORMATION FOR SEQ ID NO:3:

(a) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 199 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(i) MOLECULE TYPE: peptide

Asn Gln Arg Val Leu Gly Asn Ala Ala Gly Lys Tyr Phe Iys Val Gln
 20 25 30

Gly Glu Asp Gln Gly Gln Leu Lys Gly Met Arg Ile Phe Leu Ser Val
 35 40 45
 Phe Lys Phe Ile Lys Gly Phe Gln Leu Gln Phe Gly Ser Asn Trp Thr
 50 55 60
 Asp Val Tyr Gly Thr Arg Ser Asp Asn Phe Ile Asp Phe Leu Leu Glu
 65 70 75 80
 Asp Gly Glu His Val Ile Lys Val Glu Gly Ser Ala Val Ile Cys Leu
 85 90 95
 Thr Ser Leu Thr Phe Thr Thr Asn Lys Gly Arg Val Ala Thr Phe Gly
 100 105 110
 Val Arg Arg Gly Arg Tyr Phe Ser Asp Thr Gly Gly Ser Asp Lys His
 115 120 125
 Leu Val Thr Val Asn Gly Met His Ala Pro Gly Leu Cys Val Arg Gly
 130 135 140
 Ile Gly Phe Lys Trp Gly Asn Ile Asn Ala Asn Gly Asn Asp His Tyr
 145 150 155 160
 Asn Asn Lys Glu Asp Lys Ala Asp Asn Lys Asp Ala Asp Asn Lys Asp
 165 170 175
 Ala Asp Asn Lys Asp Asp Gly Asp Glu Asp Asp Asp Gly Asn Asp Asp
 180 185 190
 Asp Asp Gln Lys Asp Glu Ser
 195

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 166 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Leu Pro Gln Leu Glu Ala Met Leu Pro Leu Leu Ile Leu Ala Phe
 1 5 10 15
 Leu Gly Thr Pro Ala Val Leu Thr Gln Ser Arg Tyr His Gly Ser Glu
 20 25 30
 Thr Gly Lys His Phe Cys Ile Val Ala Pro Glu Gly Glu Pro Val Thr
 35 40 45
 Gly Ile Trp Ala Ser Leu Lys Asn Asn Ile Leu Ser Ser Ile Arg Leu
 50 55 60
 Lys Phe Gly Asn Asn Trp Ser Gln Glu Tyr Gly Ser Ser Gly Arg Ala
 65 70 75 80

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Ile | Glu | Val | Lys | Leu | Asn | Pro | Asp | Glu | Thr | Val | Leu | Gly | Phe | Ser |
| 85 | | | | | | | | | 90 | | | | | 95 | |
| Gly | Ser | Phe | Tyr | Ile | Phe | Met | His | Gln | Ile | Ile | Thr | Thr | Ser | Gln | |
| 100 | | | | | | | | | 105 | | | | | 110 | |
| Pro | Arg | Glu | Leu | Ile | Ile | Gly | Pro | Leu | Thr | Gly | Arg | Tyr | Val | Tyr | Thr |
| | 115 | | | | | | 120 | | | | 125 | | | | |
| Ser | Tyr | Pro | Glu | Asn | Pro | Asn | His | Val | Phe | Arg | Gly | Ile | Cys | Gly | Tyr |
| 130 | | | | | | 135 | | | | 140 | | | | | |
| Tyr | Val | Thr | Gly | Gly | Leu | Lys | Gly | Met | Arg | Tyr | Leu | Trp | Gly | Asn | Val |
| 145 | | | | | 150 | | | | | 155 | | | | 160 | |
| Asn | Gly | Thr | Cys | Thr | Glu | | | | | | | | | | |
| | | | | | 165 | | | | | | | | | | |

(2) INFORMATION FOR SEQ ID NO.5.

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 170 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Phe | Gln | Leu | Glu | Ala | Met | Leu | Pro | Leu | Leu | Ile | Leu | Ala | Phe | Leu |
| 1 | | | 5 | | | | | | 10 | | | | | 15 | |
| Gly | Thr | Pro | Thr | Val | Leu | Thr | Gln | Asp | Tyr | His | Gly | Pro | Glu | Val | Gly |
| | | | | | | | | | 20 | 25 | | | | 30 | |
| Lys | His | Ser | Cys | Thr | Ser | Ala | Pro | Glu | Gly | Lys | Asn | Ile | Thr | Ser | Ile |
| | | | | | | | | | 35 | 40 | | | | 45 | |
| Arg | Val | Phe | Leu | Gln | Gly | Arg | Ser | Ile | Val | Gly | Ile | Gln | Phe | Asn | Tyr |
| | | | | | | | | | 50 | 55 | | | | 60 | |
| Asn | Asn | Glu | Asp | Gly | Gln | Val | Tyr | Gly | Ser | Thr | Ala | Gly | Lys | Val | Met |
| | | | | | | | | | 65 | 70 | | | | 80 | |
| Val | Ala | Arg | Leu | Asn | Asn | Glu | Glu | Ser | Ile | Ile | Ala | Ala | Glu | Gly | Thr |
| | | | | | | | | | 85 | 90 | | | | 95 | |
| Tyr | Ser | Pro | Ser | Ala | Leu | Thr | Gln | Ile | Ile | Phe | Thr | Thr | Asn | Gln | Pro |
| | | | | | | | | | 100 | 105 | | | | 110 | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Gly | Thr | Gly | Ile | Lys | Ser | Ile | Leu | Phe | Leu | Trp | Gly | Thr | Glu | Asn |
| 125 | | | | | | | | | | 155 | | | | 160 | |

Ser Ser Cys Val Lys Tyr Gly His Ser Gly
165 170

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Leu Thr Leu Ala Leu Leu Gly Gly Pro Thr Trp Ala Gly Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(vii) IMMEDIATE SOURCE:

(B) CLONE: ZC12493

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGTGCTGAAA TACTTGCCTC CTCCAGGGCC ATACATCTTC

40

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(vii) IMMEDIATE SOURCE:

(B) CLONE: ZC694

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TAATACGACT CACTATAGGG

20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(vii) IMMEDIATE SOURCE:

(B) CLONE: ZC695

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GATTAGGTG ACACATATAG

19

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(vii) IMMEDIATE SOURCE:

(B) CLONE: ZC13183

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATAGAAATAG CGGTCTTGC

20

(2) INFORMATION FOR SEQ ID NO:11:

Sequence listing information is provided in the sequence description section.

(i) SEQUENCE CHARACTERISTICS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13187

(x) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCAGCCAGGC GAATACATCA

20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(x) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Glu Glu Tyr Met Pro Met Glu
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13404

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGCGAGAGGA TCCGCATGCA CCGGCCAGAG GCCATGCT

38

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(i) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

(B) CLONE: ZC13409

(x) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCGGTACCTA GCGACCCACG GGTGAGTTG CTGA

34

(2) INFORMATION FOR SEQ ID NO:15:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 55 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(i) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

(B) CLONE: ZC13407

(x) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCGGTACCTA TTCCATCGGC ATGTATTCTT CGCGACCCAC GGGTGAGTTT GCTGA

55

(2) INFORMATION FOR SEQ ID NO:16:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(i) MOLECULE TYPE: peptide

(x) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asp Tyr Lys Asp Asp Asp Lys

SEQUENCE CHARACTERISTICS.

(A) LENGTH: 18 base pairs

100

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC976

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CGTTGTAAAA CGACGGCC

18

(2) INFORMATION FOR SEQ ID NO:18:

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC447

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TAACAATTTC ACACAGG

17

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Glu Tyr Met Pro Val Asp
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 534 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

| | |
|---|-----|
| ATGCAYMGNC CNGARGCNAT GYTNYTNYTN YTNACNYTNG CNYTNYTNGG NGGNCCNACN | 60 |
| TGGGCNGGNA ARATGTAYGG NCCNGGNGGN GGNAARTAYT TYWSNACNAC NGARGAYTAY | 120 |
| GAYCAYGARA THACNGGNYT NMGNGTNWSN GTNGGNYTNY TNYTNGTNAA RWSNGTNCAR | 180 |
| GTNAARYTNG GNGAYWSNTG GGAYGTNAAR YTNGGNGCNY TNNGGNGNAA YACNCARGAR | 240 |
| GTNACNYTNC ARCCNGGNGA RTAYATHACN AARGTNTTYG TNGCNTTYCA RGNCNTYYTN | 300 |
| MGNNGGNATGG TNATGTAYAC NWSNAARGAY MGNTAYTTYT AYTYYGGNAA RYTNGAYGGN | 360 |
| CARATHWSNW SNGCNTAYCC NWSNCARGAR GGNCARGTNY TNGTNGGNAT HTAYGGNCAR | 420 |
| TAYCARYTNY TNNGGNAATHAA RWSNATHGGN TTYGARTGGA AYTAYCCNYT NGARGARCCN | 480 |
| ACNACNGARC CNCCNGTNAA YYTNACNTAY WSNGCNAAYW SNCCNGTNGG NMGN | 534 |

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: ZC13703

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

| | |
|---------------------|----|
| CCTGGGACGT GAAACTGG | 18 |
|---------------------|----|

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

length: 18 base pairs

type: cDNA

strandedness: single

topology: linear

source: ZC13703

(ii) MOLECULE TYPE: cDNA

102

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13704

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TGGAAAGGCGA CAAAGACT

18

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13465

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CGCGGGATCC GCGACCCACG GGTGA

25

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13447

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GCGCGAATTTC ATGCACCGGGC CAGAG

25

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE ZC13448

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CGCGCTCGAG CTAGCGACCC ACGGG

25

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13449

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GCGCGGATCC GGGAAAGATGT ATGGC

25

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC6583

(2) INFORMATION FOR SEQ ID NO:28

104

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: ZC5020

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CACTGGAGTG GCAACTTCCA G

21

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: ZC13735

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GGTGTAAGCT TGGACAAGAG AGATTACAAG GACGATGATG ACAAGGGTGG T

51

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 66 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: ZC14291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TGGTGCTGAA ATACTTGCCT CCTCCAGGGC CATACTCTT CCCACCACCC TTGTCATCAT

60

105

CGTCCT

66

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 44 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC14297

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AGCATTGCTG CTAAGAAGA AGGTGTAAGC TTGGACAAGA GAGA

44

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC14279

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CGTGATTTG ATGGTCGTAG TCTTCAGTGG TGCTGAAATA CTTGCCTCCT

50

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 139 base pairs
- (B) TYPE: nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

| | |
|--|-----|
| AGCATTGCTG CTAAGAAGA AGGTGTAAGC TTGGACAAGA GAGATTACAA GGACGATGAT | 60 |
| GACAAGGGTG GTGGGAAGAT GTATGGCCCT GGAGGGAGGCA AGTATTTCAG CACCACTGAA | 120 |
| GACTACGACC ATGAAATCA | 139 |

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 68 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: ZC14346

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

| | |
|---|----|
| CAGTTAATCT CACATACTCA GCAAACTCAC CCGTGGGTGCG CTAGGAATTTC TAGTATTCTA | 60 |
| GGGCTGCC | 68 |

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: ZC14218

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

| | |
|---|----|
| TGGCAAACTC TCAAAATTA TAAAAATATC CAAACAGGCA GCCCTAGAAT ACTAGAATT | 60 |
|---|----|

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

(B) CLONE: ZC14278

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

ACTAGAGGAG CCGACCACTG AGCCACCACT TAATCTCACA TACTCAGCAA A

51

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 52 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

(B) CLONE: ZC13734

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

ATCATAGAAC AGAAAAACAT TAGTTGGCAA ACTCTCAAAA ATTATAAAAA TA

52

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 154 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

ACTAGAGGAG CCGACCACTG AGCCACCACT TAATCTCACA TACTCAGCAA ACTCACCGT
GGGTCGCTAG GAATTCTAGT ATTCTAGGGC TGCCTGTTG GATATTTTA TAATTTTGA

60

120

CLAIMS

What is claimed is:

1. An isolated polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 23-178 of SEQ ID NO:2.

2. An isolated polypeptide according to claim 1, wherein said polypeptide is at least 90% identical in amino acid sequence to residues 23-178 of SEQ ID NO:2.

3. An isolated polypeptide according to claim 1, wherein said polypeptide comprises residues 27-178 of SEQ ID NO:2.

4. An isolated polypeptide according to claim 1, wherein said polypeptide comprises residues 7-178 of SEQ ID NO:2.

5. An isolated polypeptide according to claim 1, wherein said polypeptide comprises residues 1-178 of SEQ ID NO:2.

6. An isolated polypeptide according to claim 1, wherein said polypeptide is at least 1 kb in length.

7. An isolated polypeptide according to claim 1, covalently linked to a moiety selected from the group consisting of affinity tags, toxins, radionucleotides, enzymes and fluorophores.

8. An isolated polypeptide according to claim 7, wherein said moiety is an affinity tag selected from the group consisting of polyhistidine, FLAG, Glu-Glu, glutathione S transferase and an immunoglobulin heavy chain constant region.

9. An isolated polypeptide according to claim 8 further comprising a proteolytic cleavage site between said sequence of amino acid residues and said affinity tag.

10. An expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA segment encoding a polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 23-178 of SEQ ID NO:2; and

a transcriptional terminator.

11. An expression vector according to claim 10, wherein said DNA segment encodes a polypeptide is at least 90% identical in amino acid sequence to residues 23-178 of SEQ ID NO:2.

12. An expression vector according to claim 10, wherein said polypeptide encoded by said DNA segment comprises residues 27-178 of SEQ ID NO:2.

13. An expression vector according to claim 10, wherein said polypeptide encoded by said DNA segment comprises residues 7-178 of SEQ ID NO:2.

14. An expression vector according to claim 10, wherein said polypeptide encoded by said DNA segment comprises residues 1-178 of SEQ ID NO:2.

15. An expression vector according to claim 10,

...

and an immunoglobulin heavy chain constant region.

16. An expression vector according to claim 10 wherein said DNA further encodes a secretory signal sequence operably linked to said polypeptide.

17. An expression vector according the claim 10, wherein said secretory signal sequence encodes residues 7-22 of SEQ ID NO:2.

18. An expression vector according the claim 10, wherein said secretory signal sequence encodes residues 1-22 of SEQ ID NO:2.

19. A cultured cell into which has been introduced an expression vector according to claim 10, wherein said cell expresses said polypeptide encoded by said DNA segment.

20. A method of producing a protein comprising:
culturing a cell into which has been introduced an expression vector according to claim 10 whereby said cell expresses said protein encoded by said DNA segment; and
recovering said expressed protein.

21. A pharmaceutical composition comprising a polypeptide according to claim 1 in combination with a pharmaceutically acceptable vehicle.

22. An antibody that specifically binds to an epitope of a polypeptide according to claim 1.

23. A binding protein that specifically binds to an epitope of a polypeptide according to claim 1.

24. An isolated polynucleotide encoding a polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 23-178 of SEQ ID NO:2.

25. An isolated polynucleotide according to claim 24, wherein said polypeptide is at least 90% identical in amino acid sequence to residues 23-178 of SEQ ID NO:2.

26. An isolated polynucleotide according to claim 24, wherein said polypeptide comprises residues 7-178 of SEQ ID NO:2.

27. An isolated polynucleotide according to claim 24, wherein said polypeptide comprises residues 1-178 of SEQ ID NO:2.

28. An isolated polynucleotide according to claim 24, wherein said polynucleotide is selected from the group consisting of,

- a) a sequence of nucleotides from nucleotide 168 to nucleotide 704 of SEQ ID NO:1;
- b) a sequence of nucleotides from nucleotide 186 to nucleotide 704 of SEQ ID NO:2;
- c) a sequence of nucleotides from nucleotide 234 to nucleotide 704 of SEQ ID NO:2;
- d) a sequence of nucleotides from nucleotide 246 to nucleotide 704 of SEQ ID NO:2;
- e) allelic variants of a), b), c) or d); and
- f) nucleotide sequences complementary to a), b), c), d) or e).

29. An isolated polynucleotide according to claim 24, wherein said polypeptide is approximately 1 kb in length.

31. An isolated polynucleotide according to claim 24 comprising nucleotide 1 to nucleotide 534 of SEQ ID NO:20.

32. An isolated polynucleotide according to claim 24, wherein said polynucleotide is DNA.

33. An oligonucleotide probe or primer comprising 14 contiguous nucleotides of a polynucleotide of SEQ ID NO:20 or a sequence complementary to SEQ ID NO:20.

34. A method for detecting a genetic abnormality in a patient, comprising:

obtaining a genetic sample from a patient;

incubating the genetic sample with a polynucleotide comprising at least 14 contiguous nucleotides of SEQ ID NO:1 or the complement of SEQ ID NO:1, under conditions wherein said polynucleotide will hybridize to complementary polynucleotide sequence, to produce a first reaction product;

comparing said first reaction product to a control reaction product, wherein a difference between said first reaction product and said control reaction product is indicative of a genetic abnormality in the patient.

35. A DNA construct encoding a polypeptide fusion, said fusion comprising a secretory signal sequence selected from the group consisting of:

(a) amino acid residues 1-22 of SEQ ID NO:2; and

(b) amino acid residues 7-22 of SEQ ID NO:2;

wherein said secretory signal sequence is operably linked to an additional polypeptide.

36. A method for detecting zsig32 polypeptides comprising:

exposing a polypeptide containing sample to an antibody attached to a solid support, wherein said antibody binds to an epitope of a zsig32 polypeptide;

washing said immobilized antibody-polypeptide to remove unbound contaminants;

exposing the immobilized antibody-polypeptide to a second antibody directed to a second epitope of a zsig32 polypeptide, wherein the second antibody is associated with a detectable label; and

detecting the detectable label.

1/1

| | | | | | | | | | | | | | |
|----------------|----------------------------|--|-------------------|----------------|---------|---------|------------|---------|---------|---------|-------|--------|-----|
| | 10 | 20 | 30 | 40 | 50 | | | | | | | | |
| U00964_1 | MFQLEAMPLPLL | IAFLGTPAVLTQSRYHGSETGHFCIVAPEGEPTGIW | | | | 44 | | | | | | | |
| S76879_1 | MFQLEAMPLPLL | IAFLGTPTVLTQD-YHGPEVGKHSCTSAPEGKNITSIR | | | | 49 | | | | | | | |
| zsig32-bestorf | MHRPEAMLLLTLALLGGPT | --WAGKMYGPGGGKYFSTTEDYDHEITGLR | | | | 48 | | | | | | | |
| SPBP_MOUSE | -----MLLLLTLAFLASPTCRAQNVL | -GNAAGKYFYVQGEDQGQLKGMR | | | | 43 | | | | | | | |
| | 60 | 70 | 80 | 90 | 100 | | | | | | | | |
| U00964_1 | ASLKN-NILSSIRLKFGNNWSQEYGS | YGSRAEIEVKLNPD | ETVLGFSGSFY | | | 93 | | | | | | | |
| S76879_1 | VFLQG-RSIVGIQFN | YNNEDGQVY | YGSRAEIEVKLNPD | | | 98 | | | | | | | |
| zsig32-bestorf | VSVGL-LLVKS | VQVKLGDSWDV | KLGALGGNTQEVT | LPGEYITKVFVAFQ | | 97 | | | | | | | |
| SPBP_MOUSE | IFLSVFKFIKGFQLQFGS | NWTDVY | GTRSDNFIDFLLEDGEH | VIKVEGS-A | | 92 | | | | | | | |
| | 110 | 120 | 130 | 140 | 150 | | | | | | | | |
| U00964_1 | IF-MHQIIITTSQ | PRELII | IGPLG | RYVYT | SYT | PEPNPNH | VFRGIC | GYVTGG | | 142 | | | |
| S76879_1 | PSALTQI | I | FTTNQ | PRQLMV | GYVG | SSY | SSFPDDPSH | VHLKGAC | VSWRAGG | | 148 | | |
| zsig32-bestorf | AF-LRG | MV | YTSK | DRYFYFG | KLDGQ | - | ISSAYPSQEQ | QV | LVGIY | GQYQLLG | | 145 | |
| SPBP_MOUSE | VICL | TSLT | FTTNK | GRVAT | FGVRR | GRY | - | FS | DTGGSD | KHL | TVNGM | HAPGLC | 141 |
| | 160 | 170 | 180 | 190 | 200 | | | | | | | | |
| U00964_1 | LKGMR | YLWGNV | NGTCTE | ----- | ----- | | ----- | ----- | ----- | 159 | | | |
| S76879_1 | IKSIL | FLWGT | ENSSCV | KYGHSG | ----- | | ----- | ----- | ----- | 170 | | | |
| zsig32-bestorf | IKS | SIGFEW | NYPLEE | PTTEPPV | NLTYSAN | SPVGR | ----- | ----- | ----- | 178 | | | |
| SPBP_MOUSE | VRGIG | FKWGN | NINANG | NDHYNN | KEDKAD | NKDAD | NKDDG | DED | DDGND | 191 | | | |
| | 210 | | | | | | | | | | | | |
| U00964_1 | ----- | | 159 | | | | | | | | | | |
| S76879_1 | ----- | | 170 | | | | | | | | | | |
| zsig32-bestorf | ----- | | 178 | | | | | | | | | | |
| SPBP_MOUSE | DDDQKDES | | 199 | | | | | | | | | | |

Fig. 1

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/05255

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/47 A61K38/17 C07K16/18 C12Q1/68
C12N15/62 C12N15/11 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|--|-----------------------|
| X | WO 96 23080 A (ALLERGAN INC) 1 August 1996 see SEQ ID NO 1 --- | 33 |
| Y | MILLS JS ET AL: "Androgen regulated expression of a spermine binding protein gene in mouse ventral prostate" NUCLEIC ACIDS RESEARCH, vol. 15, no. 19, 1987, OXFORD GB, pages 7709-7724, XP002070923 cited in the application see figure 1 --- -/- | 35 |

Further documents are listed in the continuation of box C

Patent family members are listed in annex.

Special categories of cited documents

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "U" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is considered in combination with one or more other documents

Information for continuation of the International Search Report

Information for continuation of the International Search Report

8 July 1998

21/07/1998

Address of the ISA

U.S. Patent and Trademark Office
1401 L Street, NW
Washington, DC 20412
Tel: (202) 707-3400-3016
Fax: (202) 707-3400-3016

Address of the Office

Esben, J.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/05255

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No |
|----------|--|----------------------|
| Y | ANDEREGG RJ ET AL: "Correction of the cDNA-derived protein sequence of prostatic spermine binding protein: pivotal role of tandem mass spectrometry in sequence analysis" BIOCHEMISTRY, vol. 27, 1988, EASTON, PA US, pages 4214-4221, XP002070924 see figure 6 ----- | 35 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/05255

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
|--|------------------|-------------------------|------------|-------------------------|
| WO 9623080 A | 01-08-1996 | US 5650279 A | 22-07-1997 | AU 5295596 A 14-08-1996 |

